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(54) Title: IMMUNIZING AGAINST HIV INFECTION

(57) Abstract: A virus neutralizing level of antibodies to a primary HIV isolate is generated in a host by a prime-boost administration of antigents. The primary antigen is a DNA molecule encoding an envelop glycoprotein of a primary isolate of HIV-1 while the boosting antigen is either a non-infectious, non-replicating HIV-like particle having the envelope glycoprotein of a primary isolate of HIV-1 or an attenuated viral vector expressing an envelope glycoprotein of a primary isolate of HIV-1.

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# TITLE OF INVENTION IMMUNIZING AGAINST HIV INFECTION

#### FIELD OF THE INVENTION

[0001] The present invention relates to the field of immunology and, in particular, to methods and compositions for immunizing a host against infection with HIV.

#### **BACKGROUND OF THE INVENTION**

[0002] Human immunodeficiency virus is a human retrovirus and is the etiological agent of acquired immunodeficiency syndrome (AIDS). It is estimated that more than 33 million people have been infected with HIV world-wide as of December 1999 (Ref 1- various references are referred to in parenthesis to more fully describe the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately preceding the claims. The disclosure of these references are hereby incorporated by reference into the present disclosure).

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As the HIV epidemic continues to spread world wide, the need for an effective vaccine remains urgent. Efforts to develop such a vaccine have been hampered by several factors three of which are: (a) the extraordinary ability of the virus to mutate; (b) inability of most known specificities of anti-HIV antibodies to neutralise HIV primary isolates consistently; and (c) lack of understanding of the correlates of protective immunity to HIV infection. Over the last 10 years, several candidate HIV vaccines have been tested in primates for their immunoprotective abilities (Ref. 2). These studies suggest that both neutralising antibodies and cell-mediated immunity play a role in conferring sterilizing immunity and preventing progression towards disease (Ref 3, 4). While the correlates for immune protection against HIV-1 infection are currently unknown, an effective HIV vaccine should elicit both strong neutralising antibody and cytotoxic T lymphocyte (CTL) responses.

[0004] Envelope subunit vaccines have been shown to induce high titred humoral responses, but were inefficient in eliciting CTL responses (Ref 5). Live recombinant pox vectors have been shown to elicit very potent CTL responses, however these vectors were ineffective for generating a significant antibody

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response (Ref 6). In attempts to combine the two immunization types, several clinical trials involved a prime-boost strategy, consisting of initial viral vector immunization followed by boosts with recombinant HIV-1 envelope subunits (Ref 7, 8), have led to limited success with respect to CTL responses. Other vaccine approaches have used non-infectious, non-replicating, immunogenic virus-like particles (VLP) for immunising against HIV infection (Ref 9, 10). This type of immunogen has lead to the generation of neutralizing antibodies to a laboratory HIV-1 strain (Ref 10).

[0005] A prime-boost approach has been investigated using non-infectious VLPs to enhance HIV-specific CTL responses in mice primed with recombinant canarypox vector vCP205 encoding HIV-1gp 120 (MN strain) (Ref 11). This study showed that VLPs could boost the CTL response to the canarypox vector.

[0006] Recently, a study showing the induction of neutralizing antibodies to a HIV-1 primary isolate in chimpanzees has been reported (Ref 12). In this study, recombinant adenovirus expressing gp160 was used as the priming agent and recombinant gp120 protein was used to boost the monkeys.

[0007] There is still a need for vaccines and immunization regimes to induce both a strong CTL response as well as neutralizing antibodies to HIV primary isolates.

#### SUMMARY OF THE INVENTION

[0008] In accordance with one aspect of the present invention, there is provided a method for generating, in a host, particularly a human host, a virus neutralizing level of antibodies to a primary HIV isolate, comprising at least one administration of a priming antigen to the host, wherein the priming antigen comprises a DNA molecule encoding an envelope glycoprotein of a primary isolate of HIV, resting the host for at least one specific resting period to provide for clonal expansion of an HIV antigen specific population of precursor B-cells therein to provide a primed host, and at least one administration of a boosting antigen to the primed host to provide said neutralizing levels of antibodies, wherein the boosting antigen is selected from the group consisting of a non-infectious, non-replicating, immunogenic HIV-like particle having at least part of the envelope glycoprotein of a primary isolate of HIV and an attenuated viral

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vector expressing at least part of an envelope glycoprotein of a primary isolate of HIV.

[0009] The primary HIV isolate may be an HIV-1 isolate including from the clade B HIV-1 clinical isolate HIV-1<sub>Bx08</sub>, although any other primary HIV-1 isolate may be employed in the immunization procedures of the invention.

[0010] The DNA molecule encoding the envelope glycoprotein of a primary isolate of HIV may be contained in a plasmid vector under the control of a heterologous promoter, preferably a cytomegalovirus promoter, for expression of the envelope glycoprotein in the host, which may be a human host.

10 [0011] The vector utilized for DNA molecule immunization is novel and constitutes a further aspect of the present invention. Preferably, the vector has the identifying characteristics of pCMV3Bx08 shown in Figure 2, such identifying characteristics being the nucleic acid segments and restriction sites identified in Figure 2.

15 [0012] A priming administration of antigen may be effected in a single or in multiple administrations of the priming antigen. In the latter case, the at least one specific resting period to permit clonal expression of HIV antigen-specific population precursor B-cells may be effected after each priming administration. The at least one specific resting period may be between about 2 and 12 about 20 months.

[0013] In the embodiment where the boosting antigen is a non-infectious, non-replicating, immunogenic HIV-like particle, such particle may comprise an assembly of:

- (i) an env gene product,
- (ii) a pol gene product, and

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(iii) a gag gene product

with the particle being encoded by a modified HIV genome deficient in long terminal repeats (LTRs) and containing gag, pol and env in their natural genomic arrangement. Such particles and the manufacture thereof are described in US Patent No. 5,439,809, assigned to the assignee hereof and the disclosure of which is incorporated herein by reference. Such particles can include mutations in gag and pol to further reduce potential infectivity, as more fully described in United

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States Patent No. 6,080,408, assigned to the assignee hereof and the disclosure of which is incorporated herein by reference (WO 96/06177). In a preferred embodiment, the *env* gene is that from primary isolate BX08. The *gag* gene and *pol* gene may be those from the same primary isolate or may be chosen from those of other HIV-1 isolates, which may be primary isolates.

[0014] The non-infectious, non-replicating, immunogenic HIV-like particle may be administered in conjunction with an adjuvant. Any suitable adjuvant may be used, such as QS21, DC-chol, RIBI or Alum.

[0015] Such non-infectious, non-replicating, immunogenic HIV particle may be formed by expression from a suitable vector in mammalian cells. In accordance with an additional aspect of this invention, there is provided a vector comprising a modified HIV-genome deficient in long terminal repeats and a heterologous promoter operatively connected to said genome for expression of said genome in mammalian cells to produce the non-infectious, non-replicating and immunogenic particle, wherein at least the *env* gene of the modified HIV-genome is that from a primary isolate of HIV. The *gag* and *pol* genes of the modified HIV genome may be those from the same primary isolate or those from another isolate, which may be a primary isolate.

[0016] The vector preferably is a plasmid vector while the primary isolate preferably is BX08. The promoter may be the metallothionein promoter. The vector preferably has the identifying characteristics of plasmid p133B1 shown in Figure 3, such identifying characteristics being the nucleotide segments and restriction sites identified in Figure 3.

[0017] In the embodiment where the boosting antigen is an attenuated viral vector, the attenuated viral vector may be an attenuated avipox virus vector, particularly the attenuated canary poxvirus ALVAC. The attenuated viral vectors used herein form another aspect of the invention. The attenuated viral vector may contain a modified HIV genome deficient in long terminal repeats (LTRs), wherein at least the *env* gene is that from primary isolate BX08. The *gag* and *pol* genes of the modified genome may be those from the same primary isolate or may be chosen from other HIV isolate.

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[0018] The attenuated canarypox virus-based vector ALVAC is a plaquecloned derivative of the licensed canarypox vaccine, Kanapox, and is described in reference 19. The attenuated canary pox vector preferably has the identifying characteristics of vCP1579 shown in Figure 4, such identifying characteristics being the nucleic acid segments and restriction sites identified in Figure 4.

[0019] The at least one administration of a boosting antigen may be effected in a single administration or at least two administration of the boosting antigen.

[0020] The invention further includes compositions comprising the immunogens as provided herein and their use in the manufacture and formulation of immunogenic compositions including vaccines.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0021] The present invention will be further understood from the following description with reference to the drawings, in which:

Figure 1 shows the details of the elements of plasmid pCMVgDtat vprBx08.

Figure 2 shows the details of the elements of plasmid pCMV3Bx08.

Figure 3 shows the details of the elements of plasmid p133B1.

Figure 4 shows the details of the insertions into ALVAC (2) to provide vector vCP1579.

Figures 5A and 5B contain a representation in time-line form of the immunization regime used wherein the study groups are described in Table 1. The numbers below the lines refer to weeks.

Figure 6 shows the immunoreactivity to HIV-1 antigens of the serum diluted 1:100 from the macaques immunized with the various preparations as described in Table 1.

Figure 7 shows the immunoreactivity to HIV-1 antigens of the serum diluted 1:1000 from the macaques immunized with the various preparations as described in Table 1.

Figure 8 shows the details of the elements of pMPC6H6K3E3. Figure 9 shows the details of the elements of pMPC5H6PN.

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Figure 10 shows the details of the elements of pHIV76.

Figure 11 shows the nucleotide sequence (SEQ ID NO: 1) for the H6/HIV Pol/Nef epitope cassette in the ALVAC C5 site of vCP1579.

Figure 12 contains the nucleotide sequence of C6 region (coding strand SEQ ID NO: 16, complementary strand SEQ ID NO: 17, K3L amino acid sequence SEQ ID NO: 18, E3L amino acid sequence SEQ ID NO: 19).

#### GENERAL DESCRIPTION OF INVENTION

[0022] As noted earlier, the present invention involves administration of HTV antigens to elicit virus-neutralizing levels of antibodies against a primary HTV isolate.

[0023] A DNA construct was prepared incorporating the *env* gene from the primary isolate Bx08 under the control of the cytomegalovirus promoter and the construct, pCMV3Bx08, is shown in Figure 2. The construct pCMV3Bx08 is derived from plasmid pCMVgDtat vprBx08 seen in Figure 1. The DNA construct pCMV3Bx08 was used in a priming immunization step to a host, macaque monkeys being the animal model chosen.

[0024] Following the priming immunization step, which may be effected in one or more administrations of the DNA construct, the host is allowed to rest to provide for clonal expression of an HIV antigen specific population of precursor B-cells therein to provide a primed host.

[0025] The boosting administration is effected either with a non-infectious, non-replicating, immunogenic HIV-like particle (VLP) or an attenuated viral vector.

[0026] For this purpose, a VLP expression plasmid was constructed containing a modified HIV genome lacking long terminal repeats in which the *env* gene is derived from primary isolate BX08, wherein the modified HIV genome is under the control of a metallothionein promoter. The construct, p133B1, shown in Figure 3, was used to effect expression in mammalian cells of the non-infectious, non-replicating, immunogenic HIV-like particules, in which the *env* gene product is that from the primary isolate BX08.

[0027] In the case of the attenuated virus vector, a recombinant attenuated canarypox virus vector was constructed to contain the *env* gene from primary

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isolate BX08. The viral vector vCP1579 (Figure 4) was prepared by a variety of manipulatious from plasmid pHIV76 (Figure 10), as shown described in detail below.

[0028] These products were utilized in a boosting administration to the primed macaques. The boosting administration may be effected in one or more immunizations. In a preferred aspect of the invention, the non-infectious, non-replicating immunogenic HIV-like particles may co-administered with the DNA construct in the priming administration and the DNA construct may be coadministered with the HIV-like particles in the boosting administration.

10 [0029] Immunizations were effected in accordance with the procedure of the invention and the results obtained were compared with those obtained using other protocols according to the protocols set forth in Table 1. The immunization regimes used are shown as time lines in Figures 5A and 5B.

[0030] The results obtained following the various protocols showed that, in particular, a primary DNA vaccination in combination with a boost from either the VLP or the attenuated canarypox virus enhanced the levels of neutralizing antibodies, as indicated by the reduction of detectable p24 levels in cells infected with primary HIV isolates.

#### **Biological Deposits**

Certain vectors that are described and referred to herein have been [0031] 20 deposited with the American Type Culture Collection (ATCC) located at 10801 University Boulevard Manassas, Virginia 20110-2209, USA, pursuant the Budapest Treaty and prior to the filing of this application. Samples of the deposited vectors will become available to the public and all restrictions imposed or access to the deposits will be received upon grant of a patent based on this 25 United States patent application or the United States patent application in which they are described. In addition, the deposit will be replaced if viable samples cannot be dispensed by the Depository. The invention described and claimed herein is not limited in scope by the biological materials deposited, since the deposited embodiment is intended only as an illustration of the invention. Any 30 equivalent of similar vectors that contain nucleic acids which encode equivalent or

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similar antigens as described in this application are within the scope of the invention.

#### **Deposit Summary**

	Plasmid	ATCC	Deposit Date
5	pMT-HIV	40912	October 12, 1990
	pCMVgDtat <sup>-</sup> vpr <sup>-</sup>	209446	November 11, 1997
		EXAMPLES	•

[0032] The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

15 [0033] <u>Example 1</u>

[0034] This Example describes the construction of plasmid pCMV3BX08.

[0035] The plasmid, pCMV3BX08, contains sequence segments from various sources and the elements of construction are depicted in Figure 2.

[0036] The prokaryotic vector pBluescript SK (Stratagene) is the backbone of the plasmid pCMV3.BX08 and was modified by the replacement of the  $Amp^R$  with  $Kan^R$  gene and the deletion of the fl and the LacZ region. To achieve the desired modifications, the sequence between Ahdl (nucleotide 2,041) and Sacl (nucleotide 759) of pBluescript SK, which contains the  $Amp^R$ , fl origin and the LacZ, was deleted. A 1.2 kb Pstl fragment from the plasmid pUC-4K (Pharmacia) containing the  $Kan^R$  gene, was blunt end ligated to the Ahdl site of pBluescript SK in a counter-clockwise orientation relative to it's transcription. A 1.6 kb Sspl/Pstl DNA fragment containing the human cytomegalovirus immediate-early gene promotor, enhancer and intron A sequences (CMV) was ligated to the other end of the  $Kan^R$  gene so that the transcription from the CMV promoter proceeds in the clockwise orientation. A synthetic oligonucleotide segment containing translation initiation sequence and sequences encoding the human tissue plasminogen activator signal peptide (TPA) was used to link the

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CMV promotor and the sequences encoding the envelope gene of the primary isolate HIV-l<sub>BX08</sub>.

The envelope gene from the HIV-1 primary isolate BX08 was [0037] isolated from the plasmid pCMVgDtat vprBx08 illustrated in Figure 1. plasmid pCMVgDtat vpr Bx08 was derived from the deposited plasmid pCMVgDtat vpr, the construction of which is described in copending United States Patent Application No. 08/991,773 filed December 16, 1997, assigned to the assignee hereof and the disclosure of which is incorporated herein by reference, (WO 99/31250). The plasmid pCMVgDtat vpr Bx08 was derived by substituting the BX08 envelope sequence from clade B HIV-1 clinical isolate HIV-1<sub>BX08</sub> for the modified HIV genome sequence present in pCMVgDtat vpr. Plasmid pCMVgDtat vpr Bx08 was restricted with the restriction enzyme Xho I and made blunt ended with Klenow treatment. A Not I partial digestion was then performed and the resulting 6.3 kb fragment containing the env gene was isolated. Plasmid pCMV3 (Invitrogen) was restricted with Bam HI and made blunt ended with Klenow treatment. The plasmid pCMV3 was then restricted with Not I and the resulting 4.4 kb fragment was isolated. The 6.3 and 4.4 kb fragments were ligated together to produce plasmid pCMV3BX08 (Figure 2).

[0038] The pCMV3BX08 construct was introduced into HB101 competent cells according to manufacturer's recommendations (GibcoBRL). Correct molecular clones were identified by restriction and sequencing analysis and their expression of envelope glycoprotein was examined in transient transfections followed by Western blot analysis.

[0039] All DNAs used for immunizations were prepared using EndoFree
25 Plasmid Kit (Qiagen). For intramuscular immunizations either 3 mg or 600 μg of pCMVBX08, in 100 μl PBS was injected.

[0040] Proviral DNA for clade B HIV-1 clinical isolate HIV-1<sub>BX08</sub> originated at Transgene (Strasbourg, France) and was isolated from genomic DNA of cells infected with the virus.

30 [0041] Example 2

[0042] This Example describes the construction of plasmid p133B1.

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A Bx08 plasmid expression vector (p133B1, Figure 3) used to [0043] transfect the mammalian cells was engineered in several stages using pUC18 as the initial host plasmid. First, an 8.3-kbp fragment of HIV-1<sub>LAI</sub> provirus encoding the gag, pol and env proteins was isolated. This fragment lacked the transcription regulatory elements and long terminal repeat elements from each end of the proviral genome to ensure the virus-like particles would be replication-This fragment was linked to an inducible human type IIA incompetent. metallothionein (MTIIA) promoter (Ref 13) and also to a Simian Virus 40 polyadenylation (polyA) addition/transcription termination sequence from plasmid pSV2dhfr (Ref 14). The modified fragment was then inserted into the pUC18 host vector. The resulting deposites expression construct, named pMT-HIV, was used to transfect into African green monkey kidney (Vero) and COS monkey kidney The procedure for obtaining pMT-HIV is further described in the cells. aforementioned US Patent No. 5,439,809. Both transfected cell lines produced non-replicating virus-like particles when induced with metal ions (Ref 15).

[0044] Two further modifications were made to the provinal DNA in pMT-HIV to provide additional safety features to protect human cells against recombination events with reverse-transcribed DNA:

1) inactivation of the RNA packaging sequences; and

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20 2) deletion of a large section of the *pol* gene encoding reverse transcriptase and integrase.

[0045] To delete the first RNA packaging signal, part of the DNA corresponding to the untranslated leader sequence of the mRNA was replaced with synthetic DNA lacking a 25-bp motif corresponding to nucleotides 753-777 (the *psi* sequence). To inactivate the second RNA packaging signal, two adenosine residues within a *gag* gene zinc finger sequence were changed to thymidine residues. Each of these residue changes had the effect of replacing cysteine residues in a Cys-His array with a serine in the gene product.

[0046] The pol gene deletion was effected by replacing a 1.9-kbp fragment with synthetic DNA containing stop codons in all three reading frames. This prevented read-through translation of the residual integrase coding sequence on the 3' side of the deletion. The 1.9-kbp deletion in pol also eliminated the

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expression of reverse transcriptase and integrase enzymes. However, the deletion left intact the gene encoding the viral protease, which is both an immunogenic component of HIV-1 virus particles and allows the expression of particles with processed gag antigens closely resembling native virions (Ref 16). The protease also contains epitopes that are conserved across HIV-1 clades. The modifications described with respect to gag and pol genes are more fully described in the aforementioned United States Patent No. 6,080,408 (WO 96/06177).

Finally, the HIV-1<sub>LAI</sub> env gene within pMT-HIV was replaced with that of HIV-1<sub>Bx08</sub>. To effect this replacement, a 2440-bp fragment containing the env gene of Bx08 was amplified by polymerase chain reaction (PCR) from cells infected with this isolate. The PCR product was then used to replace the corresponding region present in pMT-HIV. However, the incoming fragment from HIV-1<sub>Bx08</sub> was 125-bp shorter than the original HIV-1<sub>LAI</sub> region owing to a deletion in the untranslated region between the env gene stop codon and the termination/polyA addition sequence. The resulting construct replaced all but eleven amino acid residues of the LAI envelope proteins gp120 and gp41. Of these eleven, only the first three differ between the LAI and Bx08 isolates, and these are all charge-conservative changes meaning the final expression vector (p133B1) encoded a nearly authentic HIV-1<sub>Bx08</sub> env protein.

20 [0048] Example 3

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[0049] This Example describes the production of HIV-like particles.

[0050] African green monkey kidney (Vero) cells were recovered and cultivated in Dulbecco's modified Eagle medium (DMEM) containing 10% v/v fetal bovine serum (FBS), referred to below as Complete Medium. At passage 141, the cells were transfected with p133B1 using the calcium phosphate method when at approximately 30% confluence. The cells were shocked with glycerol 8 hours after transfection. For this step, six 10-cm dishes containing approximately 3.0 x 10<sup>6</sup> cells each in 10.0 mL of Complete Medium were prepared. Each dish received 25.0 μg of expression vector and 2.0 μg of plasmid pSV2neo (Ref 17).

The pSV2neo contains a selectable marker gene conferring resistance to the antibiotic geneticin (G418). Two days after transfection, the cells from each dish

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were recovered by trypsinization and replated into twenty-five fresh dishes in Complete Medium supplemented with 0.5 mg/mL of G418.

In total, 394 colonies were isolated from the dishes using cloning cylinders. Each colony was recovered by trypsinization and divided into two cluster dish wells, one of the wells per clone was induced after reaching 50% to 90% confluence. Prior to induction, the wells were treated by replacing all the medium with fresh Complete Medium containing 10.0  $\mu$ M 5-azacytidine. After incubating for between 18 hours and 22 hours, the medium was removed and replaced with fresh DMEM containing 0.2% v/v FBS, 2.0  $\mu$ M CdCl<sub>2</sub> and 200.0  $\mu$ M ZnCl<sub>2</sub>. The wells were incubated for a further 20 hours to 24 hours at which time samples of the medium were removed and tested by p24 ELISA.

[0052] The twenty highest-producing clones, based on the p24 titre, were chosen and cells from the corresponding uninduced wells were sub-cultured into one T-25 and one T-150 flask per clone. Both flasks were grown to confluence.

The cells from the T-150 were recovered by trypsinization and cryopreserved at passage number 145. The cells from the T-25 were recovered by trypsinization every 3 days to 4 days and maintained up to passage 153. The cells were induced as above and samples retested by p24 ELISA at two different passages prior to passage 153.

[0053] The two highest p24 producers were chosen and were recovered by trypsinization every 3 days to 4 days up to passage 163. Samples from the clones were tested by p24 and gp120 ELISA from passage 158 and by p24 ELISA at passage 163, to assess clonal stability. The most suitable of these two cell lines, named 148 to 391, was chosen for further sub-cloning. The clone nomenclature defines the experiment number for this procedure, which was 148, and the number of the clone, which was number 391 of the original 394 isolated.

[0054] The vero cells were grown for approximately 100 h to 103 h and the medium was then replaced with growth medium containing 5-azacytidine. The bottles were then incubated for a further 20 h to 22 h, at which time the medium was replaced with serum-free medium containing CdCl<sub>2</sub> and ZnCl<sub>2</sub>. The bottles were then incubated for 29 h to 31 h, at which time the medium was harvested, pooled and stored at 2°C to 8°C prior to purification.

[0055] The next day after harvesting, the solution was clarified, concentrated and diafiltered against phosphate buffer. The concentrate was passed through a ceramic hydroxyapatite (type I) column and the run-through was collected. The run-through from two successive sublots was pooled together and pumped onto a sucrose density gradient in a continuous zonal ultracentrifuge rotor. Pseudovirion-containing fractions were collected and pooled. The pooled pseudovirion fractions were diafiltered against PBS containing 2.5% sucrose to reduce the sucrose content, concentrated and diafiltered again. The material was sterile filtered using a 0.2 µm filter. At this stage the materials was designated as a purified sub-lot and were stored at 2 to 8°C.

[0056] The adjuvants were prepared separately and filter sterilized before filling in single dose vials. QS21 was stored at -20°C.

[0057] Example 4

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[0058] This Example describes the production of recombinant pox virus vCP1579.

[0059] Recombinant pox virus vCP1579 (Figure 4) contains the HIV-1 gag and protease genes derived from the HIV-1 IIIB isolate, the gp120 envelope sequences derived from the HIV-1 Bx08 isolate, and sequences encoding a polypeptide encompassing the known human CTL epitopes from HIV-1 Nef and Pol.

[0060] Recombinant vCP1579 (Figure 4) was generated by insertion of the vector modifying sequences from pMPC6H6K3E3 (Figure 8) encoding E3L and K3L into the C6 site of recombinant vCP1566 (Figure 4). Recombinant vCP1566 was generated by insertion of an expression cassette encoding a synthetic polypeptide containing Pol CTL epitopes and Nef CTL epitopes (Figure 11) and plasmid pMPC5H6PN (Figure 9) into vCP1453 at the insertion site known as C5. Recombinant vCP1453 was generated by co-insertion of genes encoding HIV-1 env and gag/protease gene products, plasmid pHIV76 (Figure 10), into the ALVAC genome at the insertion site known as C3.

30 [0061] The construction of recombinant pox vectors containing the E3L and K3L genes has been described in United States patent 6,004,777 issued Dec 21, 1999 to Tartaglia et al. and the recombinant pox vectors describing the

insertion of HIV genes has been described in United States patent 5,766,598 issued June 16 1998 to Paoletti et al.

[0062] The locus designated C3 was used for the insertion of the HIV-1 env and gag gene sequences into the ALVAC(2) vector, and the locus designated as C5 was the insertion site for the sequences encoding the HIV-1 Nef and Pol CTL epitopes. By virtue of the C3 and C5 loci existing within the extensive inverted terminal repetitions (ITRs) of the virus genome (approximately 41 kbp), insertion into these loci results in the occurrence of two copies of the inserted HIV-1 sequences.

Briefly, expression cassette pHIV76 (Figure 10) was engineered in 10 [0063] the following manner. Plasmid p133B1 (Figure 3) containing the HIV-1Bx08 gp 160 gene was used as the starting plasmid. The 3'-end of the H6 promoter was cloned upstream of the gp160 gene and three poxvirus early transcription termination signal sequences (T<sub>5</sub>NT) were modified. This was accomplished by cloning a 2,600 bp BamHI-digested PCR fragment, containing the 3'-end of the 15 H6 promoter and the T<sub>5</sub>NT-modified HIV-1 (BX08) gp160 gene, into the BamHI site of pBS-SK. This PCR fragment was generated from four overlapping PCR fragments (a 570 bp fragment, a 140 bp fragment, a 500 bp fragment and a 1,450 bp fragment) and the oligonucleotides, RW835 (5'-ATCATCATCGGATCC CGGGGTCGCGATATCCGTTAAGTTTGTATCGTAATGAAAGTGAAGGAC 20 C-3' - SEO ID NO: 2) and RW836 (5'-ATCATCATCGGATCCCGGGGTT ATAGCAAAGCCCTTTC-3' - SEQ ID NO: 3). The 570 bp PCR fragment, containing the 3'-end of the H6 promoter and the 5'-end of the gp160 gene, was generated from the plasmid, p133B1, with the oligonucleotides, RW835 (5'-ATC ATCATCGGATCCCGGGGTCGCGATATCCGTTAAGTTTGTATCGTAATG 25 AAAGTGAAGGAGACC-3') and RW868 (5'-ATCAAGACTATAGAAGA GTGCATATTCTCTCTCATC-3'). The 140 bp PCR fragment, containing an interior portion of the gp160 gene, was generated from plasmid p133-B1 with the oligonucleotides, RW864 (5'-GCACTCTTCTATAGTCTTGATATAGTAC-3' -SEQ ID NO: 4) and RW865 (5'-AGCCGGGGCGCAGAAATGTATG 30 GGAATTGGCAC-3' - SEQ ID NO: 5). The 500 bp PCR fragment, containing an

interior portion of the gp160 gene, was generated from 133-3 with the

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oligonucleotides, RW866 (5'-ATACATTTCTGCGCCCCGGCTGGT TTTGCGATTC-3' - SEQ ID NO: 6) and RW867 (5'-GAAGAATTC CCCTCCACAATTAAAAC-3' - SEQ ID NO: 7). The 1,450 bp PCR fragment, containing the 3'-end of the gp160 gene, was generated from p133-B1 with the oligonucleotides, RW869 (5'-TGTGGAGGGGAATTCTTCTACTGTAATAC AACACAAC-3' - SEQ ID NO: 8) and RW836 (5'-ATCATCATCGGAT CCCGGGGTTATAGCAAAGCCCTTTC-3' - SEQ ID NO: 9). The 3'-end of the 570 bp PCR fragment overlaps the 5'-end of the 140 bp PCR fragment. The 3'-end of the 140 bp PCR fragment overlaps the 5'-end of the 500 bp PCR fragment. The 3'-end of the 500 bp PCR fragment overlaps the 5'-end of the 1450 bp PCR fragment. The plasmid generated by this manipulation is called pRW997.

The sequence encoding gp41 was then replaced with the sequence [0064] encoding the gp160 transmembrane (TM) region. This modification was accomplished by cloning a 200 bp MfeI-HindIII-digested PCR fragment, containing the 3'-end of the gp120 gene and the TM sequence, into the 4,400 bp MfeI-HindIII fragment of pRW997. This PCR fragment was generated from two overlapping PCR fragments (a 170 bp fragment and a 125 bp fragment) with the oligonucleotides, HIVP97 (5'-TAGTGGGAAAGAGATCTTCAGACC-3' - SEQ 10) and HIVP101 (5'-TTTTAAGCTTTTATCCCTGCCTAACT CTATTCAC TAT-3' - SEQ ID NO: 11). The 170 bp PCR fragment was oligonucleotides, HIVP97 pRW997 with the generated from TAGTGGGAAAGAGATCTTCAGACC-3' - SEQ ID NO: 12) and HIVP100 (5'-CCTCCTACTATCATTATGAATATTCTTTTTTCTCTCTGCACCACTCT-3' -SEQ ID NO: 13). The 125 bp PCR fragment was generated from pRW997 with (5'-AGAGTGGTGCAGAGAGAAAAA oligonucleotides, HIVP99 the AGAATATTCATAATGATAGTAGGAGGC-3' - SEQ ID NO: 14) and HIVP101 (5'-TTTTAAGCTTTTA TCCCTGCCTAACTCTATTCACTAT-3' - SEQ ID NO: 15). The plasmid generated by this manipulation is called pHIV71.

[0065] The H6-promoted gp120+TM gene was then cloned between C3 flanking arms, into a plasmid containing the I3L-promoted HIV1 gag/(pro) gene. This modification was accomplished by cloning the 1,600 bp NruI-XhoI fragment of pHIV71, containing the H6-promoted gp120+TM gene, into the 8,200 bp NruI-

XhoI fragment of pHIV63. The plasmid generated by this manipulation is called pHIV76 (Figure 10). Plasmid pHIV76 was used in *in vivo* recombination experiments with ALVAC (CPpp) as rescue virus to yield vCP1453.

[0066] The sequence of the nef/pol regions is shown in Figure 12 and the E3L and K3L sequences are shown in Figure 13. To generate ALVAC(2)120(BX08)GNP (vCP1579), expression cassettes consisting of the promoter/HIV-1 gene combinations were subcloned into an ALVAC donor plasmid, which were then used to insert the expression cassettes into defined sites in the ALVAC genome by *in vitro* recombination as previously described (Ref 20).

[0067] <u>Example 5</u>

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[0068] This Example describes the results of immunization regimes.

[0069] Groups of four animals (macaques) each were randomly assigned to seven vaccine groups as illustrated in Table 1. In this Table, "BX08 DNA" refers to pCMV3BX08, prepared as described in Example 1, "BX08 VLP" refers to the pseudovirions produced by expression vector p133B1 in Vero cells, as described in Example 3, and "ALVAC(2) BX08" refers vCP1579, prepared as described in Example 4. Reference (pre-bleed) sera were sampled at -6 and -2 weeks pre-vaccination. Primary immunizations with the various vaccines were given on weeks 0 and 4 with boosts on weeks 24 and 44 (Figures 5A, 5B). The vaccines were immunized intramuscularly into one quadricep of each macaque monkey.

[0070] Sera were prepared from whole-blood using SST collection tubes and analyzed using commercially available HIV-1 western blots. Groups 1, 2 and 7 showed low levels of anti-Env antibodies after the first boost (Figures 6 and 7). Based on ELISA values, the anti-env antibody levels were below 1  $\mu$ g/ml of specific IgG. High levels of anti-gag antibodies were detected in groups 1, 2, 3, 4, and 7 (Figures 6 and 7). No HIV-1 specific antibodies were detected in groups 5 and 6 (Figure 6).

The ability of the antibodies raised in the immunized monkeys to neutralize HIV-1BX08 virus in human PBMC was assayed based on the reduction of p24 levels.

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The neutralization assay was performed essentially as described in reference 18. Briefly, serum dilutions were mixed with HIV-1 BX08 and the mixtures incubated for 1 hour, then added to susceptible human PBMC cells. Titres were recorded as the dilution of serum at which p24 was reduced by 80%. Serum samples were assayed at 1:2, 1:8 and 1:32 dilution on the virus (1:6, 1:24 and 1:26 dilutions after the addition of cells). p24 levels were evaluated by p24-specific ELISA assay.

[0073] DNA vaccination on its own, group 5, and ALVAC on its own, group 6, had no monkeys showing reduction of p24 levels greater than 80%. The low DNA (600 ug) plus ALVAC, group 4, also showed no monkeys with greater than 80% reduction of p24 titres. VLP plus DNA, either high or low dose (group 1 and 2) showed enhanced reduction of p24 levels compared to VLPs alone, group 7. High dose DNA, group 3, in combination with ALVAC enhanced the ability to elicit p24 or virus neutralising antibodies over the low dose, group 4 or ALVAC alone, group 6. These results indicate that DNA vaccination in combination with VLPs or ALVAC enhanced the levels of virus neutralising antibodies as indicated by the reduction of p24 levels in the sera of the immunized monkeys.

[0074] The percentage reduction of p24 is calculated relative to the amount of p24 produced in the presence of the corresponding dilution of week 2 samples.

#### SUMMARY OF DISCLOSURE

[0075] In summary of this disclosure, the present invention provides novel immunization procedures and immunogenic compositions for generating virus neutralizing levels of antibodies to a primary HIV isolate and vectors utilized therein and for the generation of components for use therein. Modifications are possible within the scope of this invention.

Table 1 Study Design

Group number	Treatment - Week 0, 4	Treatment – Week 24,44
	3 mg BX08 DNA	3 mg BX08 DNA
1	50 μg BX08 VLP	50 μg BX08 VLP
	100 μg QS21	100 μg QS21
	600 μg BX08 DNA	600 μg BX08 DNA
2	50 μg BX08 VLP	50 μg BX08 VLP
	100 μg QS21	100 μg QS21
3	3 mg BX08 DNA	ALVAC(2) BX08 (1x10 <sup>8</sup> pfu)
4	600 μg BX08 DNA	ALVAC(2) BX08 (1x10 <sup>8</sup> pfu)
5	3 mg BX08 DNA	3 mg BX08 DNA
6	Control DNA	ALVAC(2) BX08 (1x10 <sup>8</sup> pfu)
7	50 μg BX08 VLP	50 μg BX08 VLP
	100 μg QS21	100 μg QS21

Table 2 Number of Monkeys showing > 80% reduction of p24 titre.

Group number	Week 26 Bleed	Week 44 Bleed
1	3/4	3/4
2	3/4	4/4
3	2/4	2/4
4	0/4	0/4
5	0/4	0/4
6	0/4	0/4
7	2/4	3/4

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#### **CLAIMS**

What we claim is:

1. A method for generating in a host a virus neutralizing level of antibodies to a primary HIV isolate, comprising:

at least one administration of a priming antigen to the host, wherein the priming antigen comprises a DNA molecule encoding an envelope glycoprotein of a primary isolate of HIV-1,

resting the host for at least one specific resting period to provide for clonal expansion of an HIV antigen specific population of precursor B-cells therein to provide a primed host, and

at least one administration of a boosting antigen to the primed host to provide said neutralizing levels of antibodies, wherein the boosting antigen is selected from the group consisting of a non-infectious, non-replicating, immunogenic HIV-like particle having at least the envelope glycoprotein of a primary isolate of HIV-1 and an attenuated viral vector expressing at least an envelope glycoprotein of a primary isolate of HIV-1.

- 2. The method of claim 1 wherein said primary isolate is Bx08.
- 3. The method of claim 2 wherein said DNA molecule is contained in a plasmid vector under the control of a heterologous promoter for expression of the envelope glycoprotein in the host.
- 4. The method of claim 3 wherein the promoter is the cytomegalovirus promoter.
- 5. The method of claim 4 wherein the vector has the identifying characteristics of pCMV3Bx08 shown in Figure 2.
- 6. The method of claim 1 wherein the at least one administration of a priming antigen is at least two administrations of the priming antigen.
- 7. The method of claim 6 wherein the at least one specific resting period is effected after each priming administration.
- 8. The method of claim 1 wherein the at least one specific resting period is between about 2 months to about 12 months.
- 9. The method of claim 1 wherein said non-infectious, non-replicating, immunogenic HIV-like particle comprises an assembly of:
  - (i) an env gene product,

- (ii) a pol gene product, and
- (iii) a gag gene product,

said particle being encoded by a modified HIV genome deficient in long terminal repeats (LTRs) and containing gag, pol and env in their natural genomic arrangement.

- 10. The method of claim 9 wherein the *env* gene is that from primary isolate BX08.
- 11 The method of claim 1 wherein said non-infectious, non-replicating, immunogenic HTV-like particle is administered in conjunction with an adjuvant.
- 12. The method of claim 11 wherein the adjuvant is QS21.
- 13. The method of claim 1 wherein said attenuated viral vector is an attenuated avipoxvirus
- 14. The method of claim 13 wherein the attenuated viral vector contains a modified HTV-genome deficient in long terminal repeats, wherein at least the *env* gene is that from primary isolate BX08.
- 15. The method of claim 14 wherein the attenuated avipoxvirus vector is the attenuated canary poxvirus ALVAC.
- 16. The method of claim 15 wherein the attenuated canary poxvirus vector has the identifying characteristics of vCP1579.
- 17. The method of claim 1 wherein the at least one administration of a boosting antigen is at least two administrations of a boosting antigen.
- 18. A vector, comprising a DNA sequence encoding an envelope glycoprotein of a primary isolate of HIV-1 under the control of a heterologous promoter for expression of the envelope glycoprotein in a host organism.
- 19. The vector of claim 18 wherein the vector is a plasmid vector.
- 20. The vector of claim 18 wherein said primary HIV-1 isolate is Bx08.
- 21. The vector of claim 20 wherein the promoter is the cytomegalovirus promoter.
- 22. The vector of claim 21 which has the identifying characteristics of pCMV3Bx08 shown in Figure 2.
- 23. The vector of claim 18 wherein the vector is an attenuated viral vector.

- 24. The vector of claim 23 wherein the attenuated viral vector is a attenuated avipoxvirus vector.
- 25. The vector of claim 24 wherein the attenuated avipoxvirus vector is the attenuated canary poxvirus vector ALVAC.
- 26. The vector of claim 25 wherein the attenuated viral vector has the identifying characteristics of vCP1579 shown in Figure 4.
- 27. A vector, comprising a modified HIV genome deficient in long terminal repeats and a heterologous promoter operatively connected to said genome for expression of said HIV genome in mammalian cells to produce non-infectious, non-replicating and immunogenic HIV-like particles, wherein at least the *env* gene is that from a primary isolate of HIV-1.
- 28. The vector of claim 27 wherein the vector is a plasmid vector.
- 29. The vector of claim 28 wherein the primary HIV-1 isolate is BX08.
- 30. The vector of claim 29 wherein the promoter is type IIA metallothionein promoter.
- 31. The vector of claim 30 which has the identifying characteristics of p133B1 shown in Figure 3.

Figure 1 Plasmid pCMV.Bx08.gp160

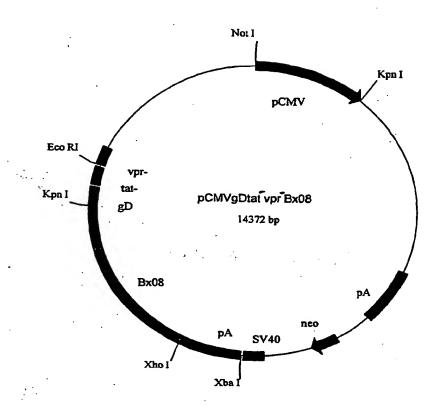


Figure 2 DNA immunization plasmid pCMV3Bx08.

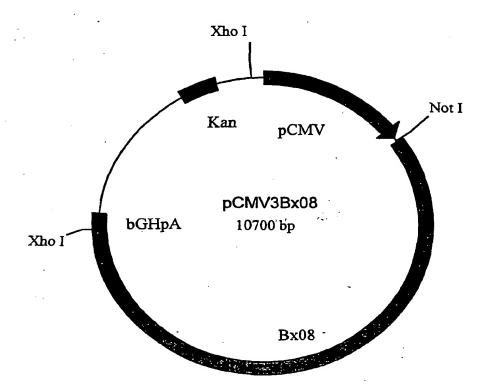


Figure 3. Pseudovirion Expression Plasmid p133B1 HIV-1 Bx08

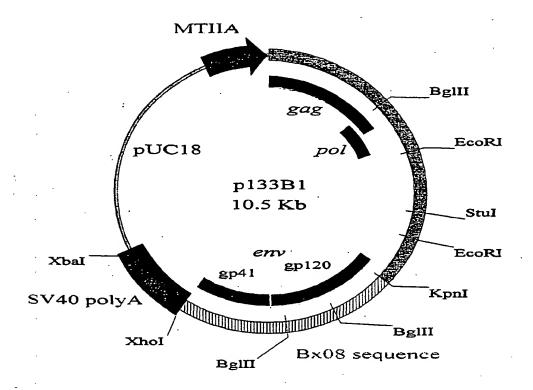
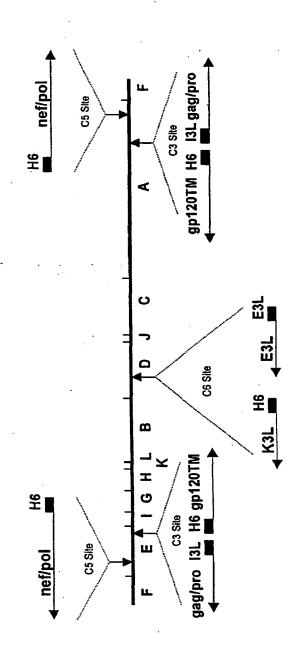
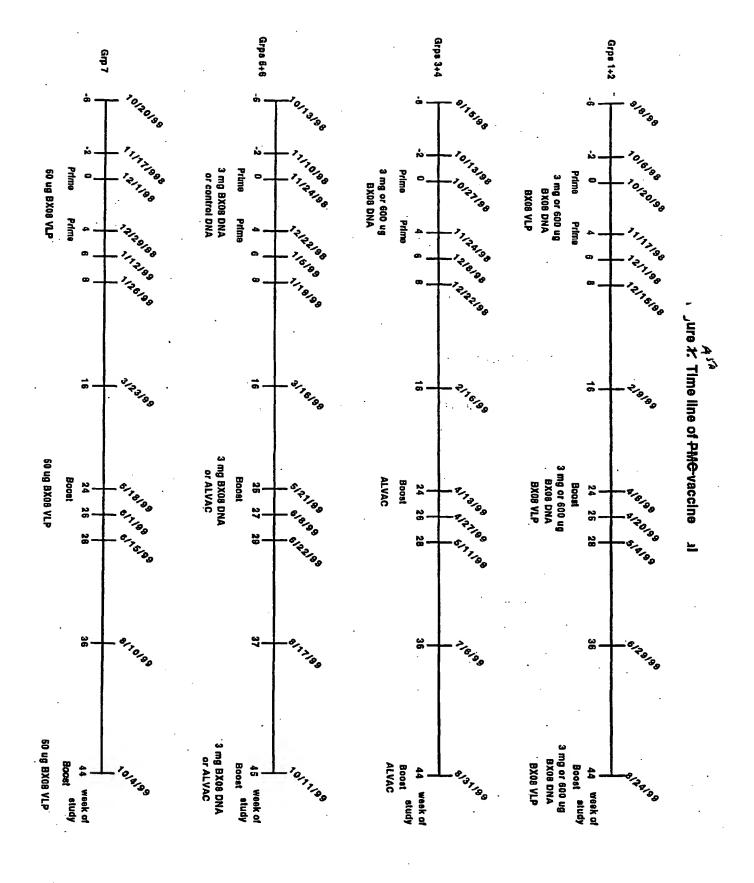


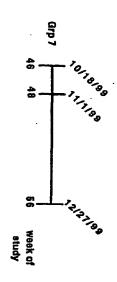
FIGURE 4

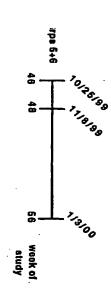
# ALVAC(2)120(BX08)GNP (vCP1579)

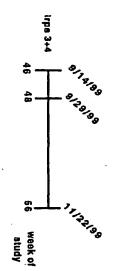
(ALVAC Xhol Restriction Map)











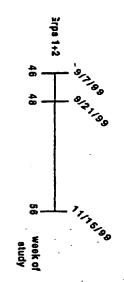
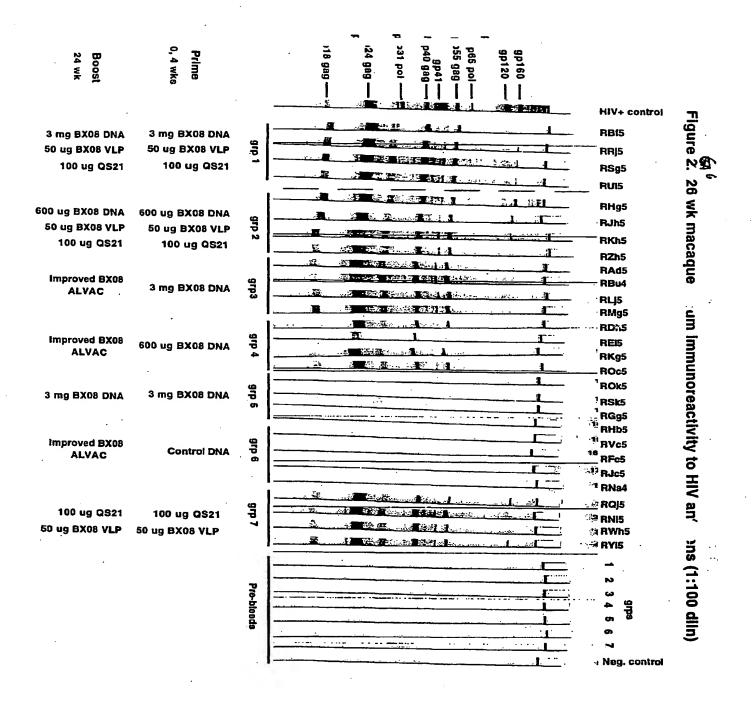
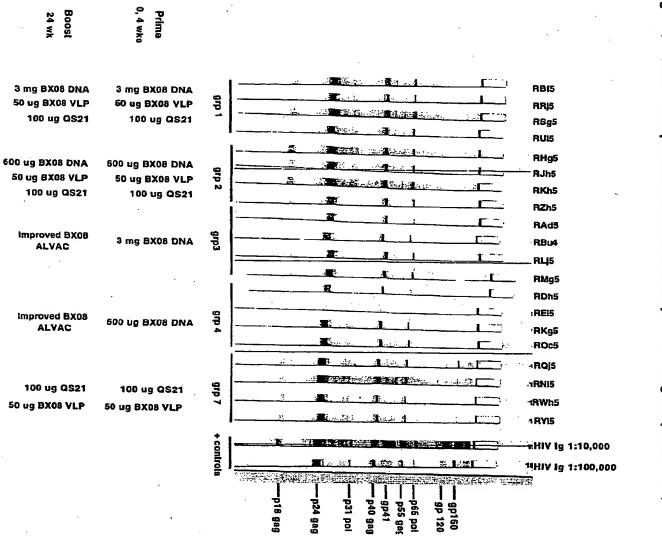
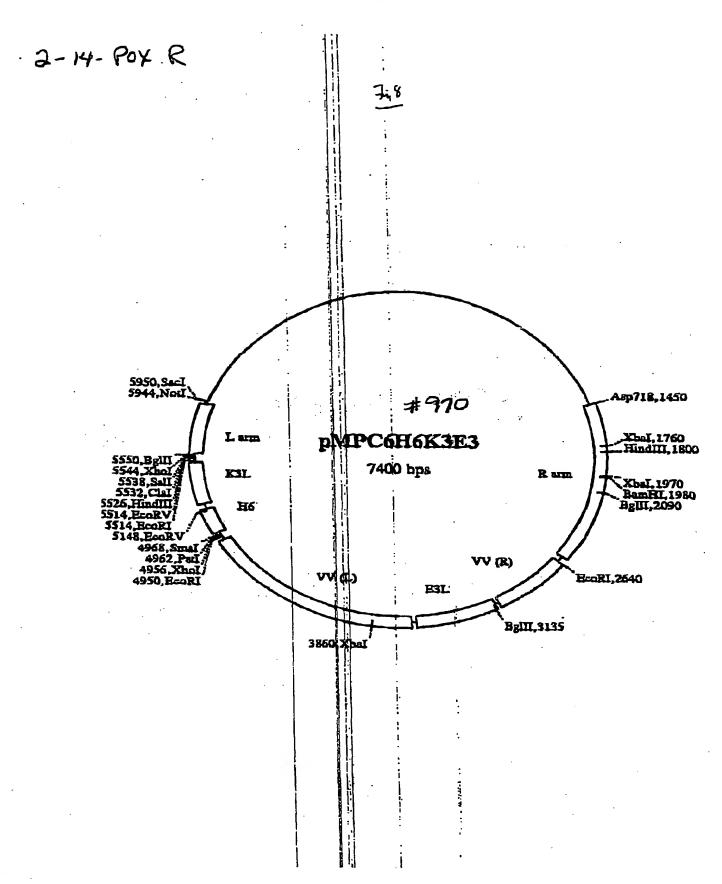
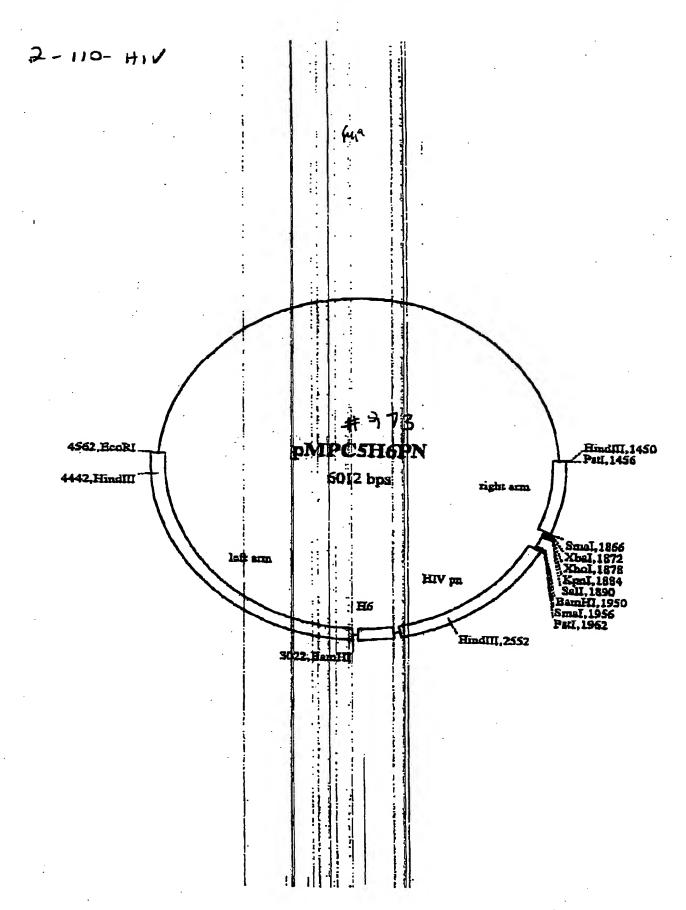


Figure **5**centinued



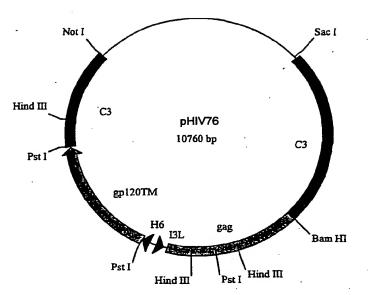






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Figure 10 Plasmid pHIV76



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Figure 11 vCP1579: H6/HIV Pol/Nef epitope cassette in ALVAC C5 site

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1 TTTTTTCAT TATTTAGAAA TTATGCATTT TAGATCTTTA TAAGCGGCCG TGATTAACTA
 61 GTCATAAAAA CCCGGGATCG ATTCTAGACT CGAGGGTACC GGATCTTAAT TAATTAGTCA
121 TCAGGCAGGG CGAGAACGAG ACTATCTGCT CGTTAATTAA TTAGGTCGAC GGATCCCCCA
181 ACAAAACTA ATCAGCTATC GGGGTTAATT AATTAGTTAT TAGACAAGGT GAAAACGAAA
241 CTATTTGTAG CTTAATTAAT TAGAGCTTCT TTATTCTATA CTTAAAAAGT GAAAATAAAT
301 ACAAAGGTTC TTGAGGGTTG TGTTAAATTG AAAGCGAGAA ATAATCATAA ATTATTTCAT
361 TATCGCGATA TCCGTTAAGT TTGTATCGTA ATGCCACTAA CAGAAGAAGC AGAGCTAGAA
421 CTGGCAGAAA ACAGAGAGAT TCTAAAAGAA CCAGTACATG GAGTGTATTA TGACCCATCA
481 AAAGACTTAA TAGCAGAAAT ACAGAAGCAG GGGCAAGGCC AATGGACATA TCAAATTTAT
541 CAAGAGCCAT TTAAAAATCT GAAAACAGGA ATGGAGTGGA GATTTGATTC TAGATTAGCA
601 TTTCATCACG TAGCTAGAGA ATTACATCCT GAATATTTTA AAAATTGTAT GGCAATATTC
661 CAAAGTAGCA TGACAAAAAT CTTAGAGCCT TTTAGAAAAC AAAATCCAGA CATAGTTATC
721 TATCAATACA TGGATGATTT GTATGTAGGA TCTGACTTAG AAATAGGGCA GCATAGAACA
781 AAAATAGAGG AGCTGAGACA ACATCTGTTG AGGTGGGGAC TTACAACCAT GGTAGGTTTT
841 CCAGTAACAC CTCAAGTACC TTTAAGACCA ATGACTTACA AAGCAGCTGT AGATCTTTCT
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1021 CCAGGACCAG GAGTCAGATA CCCATTAACC TTTGGTTGGT GCTACAAGCT AGTACCAATG
1081 ATTGAGACTG TACCAGTAAA ATTAAAGCCA GGAATGGATG GCCCAAAAGT TAAACAATGG
1141 CCATTGACAG AAGAAAAAT AAAAGCATTA GTAGAAATTT GTACAGAGAT GGAAAAGGAA
1201 GGGAAAATTT CAAAAATTGG GCCTTAATTT TTCTGCAGCC CGGGGGATCC TTTTTATAGC
1261 TAATTAGTCA CGTACCTTTG AGAGTACCAC TTCAGCTACC TCTTTTGTGT CTCAGAGTAA
1321 CTTTCTTTAA TCAATTCCAA AACAG
```

Upstream (right) flanking sequence: 1-266

VV H6 promoter: 267-390

HIV pol/nef/pol/nef/pol cassette: 391-1227 Downstream (left) flanking sequence: 1227-1345

The same of the sa

Figure 12 E3L and K3L genes in C6

10 20 30 40 50 60 70 80	90 100 110
GAGCTOROG COGCUTATON ANAGICTIAN TRACTINGGI GIAGNINGIN INGAINTING TACHANGGIN TICATATUTC CINICAN	TTC TAAAGTAGAT GATATTAATA
CTCGAGCGCC GGCGGATAGT TITCAGAATT ACTCAATCCA CATCTATCAT ATCTATAATG ATGTTTCCAT AAGTATAAAG GATAGTTT	ANG RITTCATCIA CIAIAATIAI
	200 210 220
ACTCARAGAT GATGATAGTA GATAATAGAT ACGCTCATAT AATGACTGCA AATTTGGACG GTTCACATIT TAATCATCAC GCGTTCA' TERGITICTA CTACTATCAT CTATTATCTA TGCGAGTATA TTACTGACGT TTAAACCTGC CAAGTGTAAA ATTAGTAGTG CGCAAGT	
230 240 250 260 270 280 290 300	310 320 330
ARTCTCACTA ARABGATAGO CGATGTATTT GAGAGAGATT GGACATCTAA CTACGCTARA GAARTTACAG TTATARATAA TACATAA THAGAGTGAT TTTTCTATCG CCTACATARA CTCTCTCTAA CCTGTAGATT GATGCGATTT CTTTARTGTC ARTATTTATT ANGTATT.	TGG ATTTTGTTAT CATCAGTTAT
310 330 300 300 300	420 430 440
ATTITACATA AGTACAATAA AAAGTATTAA ATAAAAATAC TTACTTACGA AAAAATGACT AATTAGCTAT AAAAACCCAG ATCTCTC TAAATTGTAT TCATGTTATT TTTCATAATT TATTTTTATG AATGAATGCT TTTTTTCGGTA TTAATCGATA TTTTTGGGTC TAGACAG	
450 460 470 480 <b>490</b> 500 510	, 520 530
TGATATCGA TICATAAAAA IT A TIG AIG TCT ACA CAT CCT TIT GIA AIT GAC AIC TAT AIA TCC TIT TGI AIA ACTATAGCIT AAGTATITIT AA I AAC TAC AGA TGI GIA GGA AAA CAT TAA CTG TAG AIA TAT AGG AAA ACA TAT	ATC AAC TOT AAT CAC TIT
ACTATAGETT ANGIATITY AN I AND THE ANGIA AND ACT IN COLOR AND ACT IN COL	DVRIVK
540 550 560 570 580 590 600	610 620
AME TIT THE AGT TIT COC THE CAG TIT ATC COT ATA THE AND ATE TOT ATC CAT ATG CAT CIT AND ATG TOA ANA GGG ATG GTC ANA TAG GGA THI AND THE TAI AGA TAG GTA TAC GTA GAA TIG TGA GAG	ACG GIT CTA TCG AAG TCT
V K V T K G V L K D R Y E V Y R D M H M K V S E	
630 640 650 660 670 680 690 GTG AGG ATA GTC ARA AAG ATA ART GTA TAG AGC ATA ATC CTT CTC GTA TAC TCT GCC CTT TAT TAC ATC GCC	700 710
CAC TCC TAT CAG ITT TIC TAT THA CAT ATC TCG TAT TAG GAA GAG CAT ATG AGA CGG GAA ATA ATG TAG CGG  CH P Y D F L Y I Y L A Y D K E Y V E G K I V D G	GCG TAA CCC GTT GCT TAT
720 730 740 750 760 770 780 790	
720 730 740 750	800 810
ACA ARA TGC ARG CAT ACG ATACAMACTT AACGGATATC GCGATAATGA AATAATITAT GATTATTCT CGCTTTCAAT TTAAC TGT TTT ACG TTC GTA TGC TATGTTTGAA TTGCCTATAG CGCTATTACT TTATTAMATA CTAATAMAGA GCGAAAGTTA AATTG <c a="" f="" l="" n<="" td=""><td>ACAAC CCTCAAGAAC</td></c>	ACAAC CCTCAAGAAC
ACA ANA TOC ANG CAT ACG ATACANACTT ARCGGATATC GCGATAATGA ANTAATTTAT GATTATTCT CGCTTTCAAT TTAAC TOT TIT ACG TIC GAT TGC TATGTTTGAA TIGCCTATAG CGCTATTACT TTATTAAATA CTAATAAAGA GCGARAGTTA AATTG <c a="" f="" l="" m<="" td=""><td>ACAAC CCTCAAGAAC</td></c>	ACAAC CCTCAAGAAC
ACA ANA TOC ANG CAT ACG ATACANACTT MACGGATATE GCGATAATGA MATAATITAT GATTATTICT CGCTTTCAAT ITAAC TOT TIT ACG TIC GIA TGC TATGITIGAA TIGCCTATAG CGCTATTACT TIAITAMATA CTAMTAMAGA GCGARAGITA MATTG CC F A L M.	PACAAC CCTCAAGAAC PTGTTG GGAGTTCTTG  900 910 920 PATC ACTAATTAAT TAACCCGGGC
ACA ANA TOC ANG CAT ACG ATACANACTT ANCOGATATE GEGATANTGA ANTANTITAT GATTATTICT CGETTICANT TITANE TOT TIT ACG TIC GIA TGC TATGOTTIGAN TIGECTATAG CGETATTACT TIATTANATA CTANTANAGA GEGARAGITA ANTIGEC F A L M.	PACAAC CCTCAAGAAC PTGTTG GGAGTTCTTG  900 910 920 PATC ACTAATTAAT TAACCCGGGC
ACA ANA TOC ANG CAT ACG ATACANACTT MACGGATATE GCGATAATGA NATAATTATA GATTATTICT CGCTITCAAT TITAAC TOT TIT ACG TIC GIA TGC TATGTTIGAA TIGCCTATAG CGCTATTACT TIATTAAATA CTAATAAAGA GCGANAGTTA AATTG  CC F A L M  B20 B30 840 850 860 870 880 890  CTITGTATTI ATTITCACTT TITAAGTATA GAATAAAGAA AGCTCTAATT AATTAATGAA CAGATIGTIT CGTTITCCCC TIGGCGI GAAACATAAA TAAAAGTGAA AAATTCATAT CTTATTCTT TCGAGATTAA TIAATTACTT GTCTAACAAA GCAAAAGGGG AACCGCR  930 940 950 960 970 980 990 1000	900 910 920 PATC ACTANTANT TAACCOGGC ATAG TGATTANTA ATTGGGCCCG  1010 1020 1030 AACG GGAACAGGGT TTGTTGATTC
ACA ANA TOC ANG CAT ACG ATACANACTT AACGGATATC GCGATAATGA NATAATTITAT GATTATTICT CGCTITCAAT TITAACT TIT ACG TIT GOA TGC TATGTTIGAA TIGCCTATAG CGCTATTACT TIATTAAATA CTAATAAAGA GCGAAAGTTA AATTGCC F A L M.  B20 B30 B40 B50 B60 870 880 890  CTITGTATTI ATTITCACIT TITAAGTATA GAATAAAGAA AGCTCTAATT AATTAATGAA CAGATTGTIT CGTTTTCCC TIGGCGI GAACATAAA TAAAAGTGAA AAATTCATAT CTTATTCTT TCGAGATTAA TTAATTACTT GTCTAACAAA GCAAAAGGGG AACCGCA  930 940 950 960 970 980 990 1000 TGCAGGTCGA GGAATTCAAC TATTCATTT GTATTCACTT GTCTAACACAT GACCATTACT AACGTAGAAT GTATAGGAAG AGATGTTAACGTCGAGCT CCTTAAGTTG ATATAGCTGT ATAAAGTAAA CATATGTGTA TIGGTAATGA TTGCATCTTA CATATCCTTC TCTACAT	900 910 920 PATC ACTANTANT TAACCOGGC ATAG TGATTANTA ATTGGGCCCG  1010 1020 1030 AACG GGAACAGGGT TTGTTGATTC
ACA ANA TOC ANG CAT ACG ATACAMACTT MACGGATATE GCGATAATGA MATAATTITAT GATTATTICT CGCTITCMAT TITAAC TOT TIT ACG TIC GIA TGC TATGITIGAA TIGCCTATAG CGCTATTACT TIATTAMATA CTAATAMAGA GCGAMAGITA AMTIG CC F A L M.  820 830 840 850 860 870 880 890 CITIGIATTI ATTITCACTI TITAAGTATA GAATAAAGAM AGCTCTAATT MATTAATGAM CAGATIGITI CGTTITCCCC TIGGCGI GAAACATAAM TAAMAGTGAM AMATTCATAT CTIMITCTT TCGAGATTAAT TAATTAATGAM GCAMAAGGGG MACCGCM 930 940 950 960 970 980 990 1000 3 TGCAGCTCGA GGAATTCAAC TATATCGACA TATTTCATTT GTATACACAT AACCATTACT AACGTAGAMT GTATAGGGAAG AGATGTA ACGTCGAGCT CCTTAAGTTG ATATAGCTGT ATAMAGTAMA CATATGTGTA TIGGTAATGA TTGCATCITA CATATCCTTC TCTACAT	PACABAC CCTCAAGAAC PROTTE GGAGTICTTG  900 910 920  CATC ACTAATTAAT TAACCCGGGC ATAG TGATTAATTA ATTGGGCCCG  1010 1020 1030  AACG GGAACAGGGT TTGTTGATTC TTGC CCTTGTCCCA AACAACTAAG  1120 1130 1140  GTAA GATGATGTTA ACTATGTGAT
ACA ANA TOC ANG CAT ACG ATACANACTT AACGGATATE GCGATAATGA NATAATITAT GATTATTICT CGCTITCART TTAAC TOT TIT ACG THE GIA TGC TATGITIGAA TIGCCTATAG CGCTATACT THATAAATA CTAATAAAGA GCGARAGITA AATTG CC F A L M  820 830 840 850 860 870 880 890  CTITGTATIT ATTITCACTT TITAAGTATA GRATAAAGAA AGCTCTAATT AATTAATGAA CAGATTGITT CGTTITCCC TIGGCGI GAAACATAAA TAARAGIGAA RAATTCATAT CITATITCTT TCGAGATTAA TTAATTACTT GTCTAACAAA GCAAAAGGGG AACCGCC  930 940 950 960 970 980 990 1000 3  TGCAGCTCGA GGAATTCAAC TATATCGACA TATTTCATTT GTATACACAT AACCATTACT AACGTAGAAT GTATAGGAAG AGATGTA ACGTCGAGCT CCTTAAGTTG ATATAGCTGT ATAAAGTAAA CATATGGTA TIGGTAATGA TIGCATCTTA CATATCCTTC TCTACAAT  1040 1050 1060 1070 1080 1090 1100 1110 3  GCAAACTATT CTAATACATA ATTCTTCTGT TAATACGTCT TGCACGTAAT CTATTATAGA TGCCAAGATA TCTATTATAAT TATTTTC CGTTTGATAA GATTATGTAT TAAGAAGACA ATTATGCAGA ACGTGCATTA GATAATACTT ACGGTTCTAT AGATATATTA ATAAAAAG	PACABAC CCTCAAGAAC PROTTE GGAGTICTTG  900 910 920  CATC ACTAATTAAT TAACCCGGGC ATAG TGATTAATTA ATTGGGCCCG  1010 1020 1030  AACG GGAACAGGGT TTGTTGATTC TTGC CCTTGTCCCA AACAACTAAG  1120 1130 1140  GTAA GATGATGTTA ACTATGTGAT
ACA ANA TOC ANG CAT ACG ATACAMACTI MACGGATATE GCGATAATGA MATAATITAT GATTATTICT CGCTITCAAT ITAAC TOT TIT ACG TIC GIA TGC TATGITIGAA TIGCCTATAG CGCTATACT TIATAAATA CTAATAAAGA GCGAAAGTTA MATGC C F A L M  820 830 840 850 860 870 880 890  CTITGTATIT ATTITCACTI TITAAGTATA GAATAAAGAA AGCTCTAATI MATAATGAA CAGATTGITI CGTTITCCC TIGGCGI GAAACATAAA TAAMAGTGAA RAATTCATAT CITATTCTI TCGAGATTAA TTAATTACTI GTCTAACAA GCAMAAGGGG AACCGCC  930 940 950 960 970 980 990 1000 1  TGCAGCTCGA GGAATTCAAC TATATCGACA TATTTCATT GTATACACAT AACCATTACT AACGTAGAAT GTATAGGAAG AGATGTA ACGTCGAGCT CCTTAAGTTG ATATAGCTGT ATAAAGTAAA CATATGTTA TIGGTAATGA TIGCAACTTA CATATCCTTC TCTACAAT  1040 1050 1060 1070 1080 1090 1100 1110 2  GCAAACTATT CTAATACATA ATTCTTCTGT TAATACGTCT TGCACGTAAT CTATTATAGA TGCCAAGGATA TCTATATAAT TATTTTC CGTTTGATAA GATTATGTAT TAAGAAGACA ATTATGCAGA ACGTGCATTA GATAATACTA ACGGTTCTAT AGATAATTA ATAAAAG	900 910 920 TATE ACTANTANT TANCEGGGE ATAG TGATTANTA ATTGGGCCCG  1010 1020 1030 AACG GGAACAGGGT TTGTTGATTC FTGC CCTTGTCCCA AACAACTAAG  1120 1130 1140 GTAA GATGATGTTA ACTATGTGAT EATT CTACTACAAT TGATACACTA  1230 1240 1250 TCCT CAAAAAATAT ATTGGCATAT
ACA ANA TOC ANG CAT ACG ATACANACTT ANCEGATATE GCGATAATGA NATAATTITAT GATTATTICT CGCTITCART ITAAC TOT TIT ACG TIC GIA TGC TATGITIGAA TIGCCTATAG CGCTATACT TIATAAATA CTAATAAAGA GCGANAGTTA ANTIC C F A L M  820 830 840 850 860 870 880 890  CTITGIATIT ATTITCACTT TITAAGTATA GAATAAAGAA AGCTCTAATT AATTAATGAA CAGATTGITT CGTTTTCCCC TIGGCGG GAAACATAAA TAANAGTGAA AAATTCATAT CTINITICTI TCGAGATTAA TAATTAATGAA CAGATTGITT CGTTTTCCCC TIGGCGG 930 940 950 960 970 980 990 1000 1  TGCAGGCTCGA GGAATTCAAC TATATCGACA TATTTCATTT GTATACACAT AACCATTACT AACGTAGAAT GTATAGGAAG AGATGITA ACGTCGAGCT CCTTAAGTTG ATATAGCTGT ATAAAGTAAA CATATGIGTA TIGGTAATGA TIGCAACTTA CATATCCTTC TCTACAT  1040 1050 1060 1070 1080 1090 1100 1110 1  GCGAAACTATT CTAATACATA ATTCTTCTGT TAATACGTCT TGCACGTAAT CTATTATAGA TGCCAAGATA TCTATATATAT TATTTTTC CGTTTGATAA GATTATGTAT TAAGAAGAAA ATTATGCAGA ACGTGCATTA CATATACTA ACGGTTCTAT AGATATATTA ATAAAAAC  1150 1160 1170 1180 1190 1200 1210 1220  CTATATAAAGT AGTGTAATAA TTCATGATAT TCGATATATG TTCCAACTCT GTCTTTGTGA TGTCTAGGTT CGTAATATCT ATAGGAACGGATATATTA AGATATATTA ATAAAAC  CTATATAAAGT AGTGTAATAA TTCATGATAT TCGATATATG TTCCAACTCT GTCTTTGTGA TGTCTAGGTT CGTAATATCT ATAGGAACGGATATATCT AGATATATCT ATAGGAACATATTCAACATTATT AAGTAACATAA AGCTATATAC AAGGTTGAGA CAGAACACT ACAGATCAAA GCATTATATCA ATATAGGAT  CTATATAAAGT AGTGTAATAA TTCATGATAT TCGATATATC TACCACTCT GTCTTTGTGA TGTCTAGGTT CGTAATATCT ATAGGAT CTATATATAT TCACATTATT AAGTACATAA AGCTATATAC AAGGTTGAGA CAGAACACT ACAGGATCAAA GCATTATATAGA TATCTGTT  1260 1270 1280 1290 1300 1310 1310 1320 1330	900 910 920 PATE ACTANTANT TAACCOGGC ATAG TGATTAATTA ATTGGGCCG  1010 1020 1030 AACG GGAACAGGGT TTGTTGATTC ATTGC CCTTGTCCCA AACAACTAAG  1120 1130 1140 GTAA GATGATGTTA ACTATGTGAT CATT CTACTACAAT TGATACACTA  1230 1240 1250 TCCT CAARAAATAT ATTCGCATAT AGGA GTTTTTTATA TAACCGTATA
ACA ANA TEC ANG CAT ACG ATACANACTT AACGGATATE GCGATAATGA ANTANTITAT GATTATTICT CECTITCAAT THANGTOT TIT ACG TIC GIA TGC TATGITIGAA TIGCCTATAG CGCTATTACT THATTAAATA CTAATAAGA GCGAAAGTTA AATGCCC F A L M.  820 830 840 850 860 870 880 890  CITIGTATIT ATTITCACTT TITAAGTATA GAATAAAGAA AGCTCTAATT AATTAATGAA CAGATIGTIT CGTTITCCCC TIGGCGI GAAACATAAA TAAAAGTGAA AAATTCATAT CTTATTCTT TCGAGGATTAA TAATTAATGAA CAGATIGTIT CGTTITCCCC TIGGCGI GAAACATAAA TAAAAGGGAA AAATTCATAT CTTATTCTT TCGAGGATTAA TAATTAACTT GTCTAACAAA GCAAAAGGGG AACCGCA ACGTCGAGGCT CCTTAAGTTGA ATATACGCAA TATTCATAT GTATACACAT AACCATTACT AACGTAGAAT GTATAGGAAG AGATGTA ACGTCGAGCT CCTTAAGTTG ATATACGCTA ATATACGTATA CATATCCTTC TCTACAAT ACGTCGAGAT TCTAATACATA ATTCTTCTT TAAAAGTAAA CATATGGTAT CTAATACGAT ACGTCGAAT TCTAATACATA ATTCTTCTTT TAAAAGTAAA ACGTCGAAT CTAATACATA ACGTTCATA TATTTTCCTTTTGATAA GAATAATATAT TAATTTTTCCTTTGATAAA GAATAATGTAA ATTCTTCTTTTTTTTTT	900 910 920  PATC ACTANTANT TAACCOGGC ATAG TGATTANTA ATTGGGCCG  1010 1020 1030  AACG GGAACAGGGT TTGTTGATTC FTGC CCTTGTCCCA AACAACTAAG  1120 1130 1140  GTAA GATGATGTTA ACTATGTGAT CATT CTACTACAAT TGATACACTA  1230 1240 1250  TCCT CAAAAAATAT ATTCGCATAT ACGG GTTTTTTATA TAAGCGTATA ACGA GTTTTTTATA TAAGCGTATA 1340 1350 1360  GATA TAGTTTTTGA CACTATCTC
ACA ANA TOC ANG CAT ACG ATACANACTT NACGGATATE GCGATAATGA ANTANTITAT GATTATTICT CGCTTCAAT TTAACT TOT TIT ACG TIC GIA TGC TATGITIGAA TIGCCTATAG CGCTATTACT TIATAAATA CTAATAAAGA GCGAAAGITA AATGCC F A L M.  820 830 840 850 860 870 880 890  CITIGTATIT ATTITCACTI TITAAGTATA GAATAAAGAA AGCTCTAATI AATTAATGAA CAGATIGITI CGTTITCCCC TIGGCGI GAAACATAAA TAAAAAGTGAA AAATTCATAT CTTATTCTT TCGAGGATTAA TTAATTACTI GTCTAACAAA GCAAAAGGGG AACCGCA  930 940 950 960 970 980 990 1000 1  TGCAGCTCGA GGAATTCAAC TATATCGACA TATTTCATTI GIATACACAT AACCATTACT AACGTAGAAT GTATAGGAAG AGATGTA ACGTCGAGCT CCTTAAGTTG ATATAGGACA TATATCATAT TATGATATA TTGCATATCA TACCATTACT ACGTTAGAT CATATCCTTC TCTACAAT  1040 1050 1060 1070 1080 1090 1100 1110 1  GCAAACTATT CTAATACATA ATTCTTCTGT TAATACGTCT TGCACGTAAT CTATTATAGA TGCCAAGATA TCTATATAAAT TATTTTTC CGTTTGATAA GATTATGTAT TAAGAAGACA ATTATGCAGA ACGTGCATTA GATAATATCT ACGGTTCTAT AGATATATTA ATAAAAAC  1150 1160 1170 1180 1190 1200 1210 1220 1  CTATATAAGT AGGTGAATAA TTCATGTATT TCGATATATG TTCCAACTCT GTCTTTGTGA TGCTCTAGTTT CGTATATATA ATAAAAAC  CTATATAAGT AGGTGAATAA TTCATGTATT TCGATATATG TTCCAACTCT GTCTTTGTGA TGCTCTAGTTT CGTATATACT ATAAAAAC  1150 1160 1270 1280 1290 1300 1310 1320 1330 1330 1310 1320 1330 1310 1320 1330 1310 1320 1330 1310 1320 1330 133	900 910 920 FATC ACTANTANT TAACCOGGC ATAG TGATTANTTA ATTGGGCCCG ATAG TGATTANTTA ATTGGGCCCG ATAG GGAACAGGGT TTGTTGATTC ACTGGCCCTTGTCCCA AACAACTAAG ACG GGAACAGGGT TTGTTGATTC ACTGGCCCA TAGATATA ACTATGGAT CATT CTACTACAAT TGATACACTA 1230 1240 1250 TCCT CAAAAAATAT ATTCGCATAT AGGA GTTTTTTATA TAACCGTATA AGGA GTTTTTTATA TAACCGTATA AGGA TAGTTTTTGA CACTATCTTC CTAT ATCAAAAACT GTGATAGAAG 1450 1460 1470
ACA ANA TEC ANG CAT ACG ATACANACTT AACGGATATE GCGATAATGA ANTANTITAT GATTATTICT CECTITCAAT TITAACT TOT TIT ACG TIC GIA TGC TATGITIGAA TIGCCTATAG CGCTATTACT TIATTAAATA CTAATAAAGA GCGAAAGTTA AATTGCC F A L M.  820 830 840 850 860 870 880 890  CITIGIANTI ATTITCACTI TITAAGTATA GAATAAAGAA AGCTCTAATI AATTAATGAA CAGATIGITI CGTTITCCCC TIGGCGI GAAACATAAA TAAAAGGGGA AAATCATAT CTIATTCTI TCGAGGATTAA TAATTAATGAA CAGATIGITI CGTTITCCCC TIGGCGI GAAACATAAA TAAAAGGGA AAATCATAT CTIATTCTI TCGAGGATTAA TAATTAACTA GCGAAAAGGGG AACCGCA ACGTCGAGGCT CCTTAAGTTG ATATACGACA TATTTCATTI GIATACACAT AACCATTACT AACGTAGAAT GTATAGGAAG AGATCACACACACACACACACACACACACACACACACACA	900 910 920  PATC ACTANTANT TARCCOGGC ATAG TGATTANTA ATTGGGCCG  1010 1020 1030  AACG GGAACAGGGT TTGTTGATTC TTGC CCTTGTCCCA AACAACTAAG  1120 1130 1140  GTAA GATGATGTTA ACTATGTGAT CATT CTACTACAAT TGATACACTA  1230 1240 1250  TCCT CAAAAAATAT ATTCGCATAT AAGGG GTTTTTTATA TAACGGTATA AAGAA TTGTTTTATA TAACGGTATA 1340 1350 1360  GATA TAGTTTTTGA CACTATCTC CTAT ATCAAAAACT GTGATAGAAG  1450 1460 1470  CTGG ACATAATTCA TCTATTATAC
ACA ARA TGC ANG CAT ACG ATACANACTT NACGGATATC GCGATAATGA ARTAATTTAT GATTATTCT CGCTTTCAAT TTAAC TOT TIT ACG TTC GTA TGC TATGTTGAA TTGCCTATAG GCGATATCT TTATTAAATA CTAATAAAGA GCGATAGTTA ARTO CC F A L N.  820 830 840 850 860 870 880 890  CTTTGTATT ATTTTCACTT TITAAGTATA GAATAAAGAA AGCTCTAATT AATTAATGAA CAGATTGTT CGTTTTCCC TTGGGGT GAAACATAAA TAAAAGTGAA AAATTCATAT CTTATTTCTT TCGAGATTAA TTAATTCACT GTCTAACAAA GCAAAAGGGG AACCGCA  930 940 950 960 970 980 990 1000 3  TGCAGCTCGA GGAATTCAAC TATATCGACA TATTTCATT GTATACACAT AACCATTACT AACGTAGAAT GTATAGGAAG AGATGTAA ACCTCGAGCT CCTTAAGTTG ATATAGCTGT ATAAAGTAAAA CATATGTGTA TTGGTAATAGA TTGCACTTC CCTAAGTAGAAA ACCTCGAGCT CCTTAAGTTG ATATAGCTGT ATAAAGTAAAA CATATGTGTA TAGGTAATGA TTGCACTTC TCTACAAT  1040 1050 1060 1070 1080 1090 1100 1110 3  GCAAACTATT CTAATACATA ATTCTTCTGT TAATACGTGT TGCACGTAAT CTATATAGA TGCCAAGATA TCTATATAAT TATTTTC CGTTTGATAA GATTATGTAT TAAGAAGACA ATTATGCAGA ACGTGCATTA GATAATATCA AGGATTAATATA ATAAAAAC  1150 1160 1170 1180 1190 1200 1210 1220 3  CTATATAAGT AGTGTAATAA TCCATGTATT TCCATATATG TTCCAACTCT GTCTTTGTGA TGCTAAGTTT CGTAATATCT ATAGCAC GATATATCA TCACATTATT AAGTACATA AGCTATATCA AAGGTTGAGA CAGAAACACT ACAGATCAAA GCATTATAGA TATCGTA ATTCCCAAGT CTTCAGTTCT ATCTCTAAAA AAACCTTCAA AGGTTGAGAT ATAAAAACACT ACAGATCAAA GCATTATAGA TATGAGAT  ATTCCCAAGT CTTCAGTTCT ATCTCTAAAA AAACCTTCAA CGTATGGGAT ATAAAAACACT ACAGATCAAA GCATTATACA ATGAGAT TAAGGGGTTCA GAAGGCAAATAT ATCTCTAAAA AAACCTTCAA CGGATACCTTA TATTATAATA TAAAATGAGAA TAAAAAGAGATTAA TTCATATTAA TAGAGATTAAT TATGAGATTAA TAGAGATTAA TAGAGATTAAT TATGAGATTAA TAGAGATTAAT TATGAGATTAA TAGAGATTAA TAGAGATTATA TAGAGATTAA TA	900 910 920  TATE ACTANTANT TARCEGGGE ATAG TGATTANTA ATTGGGCCG  1010 1020 1030  AACG GGAACAGGGT TTGTTGATTC FTGC CCTTGTCCCA AACAACTAAG  1120 1130 1140  GTAA GATGATGTTA ACTATGTGAT EATT CTACTACAAT TGATACCTA  1230 1240 1250  TCCT CAAAAAATTA ATTGGCATAT ACGA GTTTTTTATA TAAGCGTATA 1340 1350 1360  GATA TAGTTTTGA CACTATCTC CTAT ATCAAAAACT GTGATAGAAG  1450 1460 1470  CTGG ACATAATTCA TCTATTATAC GACC TGTATTAAGT AGATAATATG
ACA ANA TGC ANG CHT ACG ATACANACTT NACGGATATE GCGATANTGA ANTATTAT GATTATTCT CGCTTTCANT TTAMOUT TOT TIT ACG TTC GTA TGC TATGCTATGA TEGCCTATAG GCGATANTGT TTATAMATA CTAMTANAGA GCGANAGTTA ANTOC C F A L M.  820 830 840 850 860 870 880 890  CTITGITATT ATTITCACTT TITANGTATA GAATANAGA AGCTCTAATT ANTANAGA CAGATIGITI CGTTTTCCC TIGGGG GAAACATAAA TAANAGTGAA AAATCATAT TCGAGATTAA TTAATTACTT GCCTAACAAA GCAAAAGGGG AACCGCA  930 940 950 960 970 980 990 1000 3  TGCAGCTCGA GGAATTCAAC TATATCGACA TATTTCATT GTATACACAT AACCATTACT AACGTAGAAT GTATAGGAAG AGATGTA ACCTCGAGCT CCTTAAGTTG ATATAGCAGT ATAAAGTAAA CATATGGTA TIGGTAATGA TIGCATCTTA CATATCCTTC TCTACAT  1040 1050 1060 1070 1080 1090 1100 1110 1110 GCAAACTAT CTAATACATA ATTITCTCTG TATATCCAGT TGCACGTAAT CTAATATAGA TGCCAAGATA TCTATATATAT TATTTTC CGTTTGAATA GATATATGATA TAAGAGAACA ATTATGCAGA ACGTGCATA GATAATATCT ACGGTTCTAT AGATATATTA ATAAAAACGTTTAAAAGTAAA GATATATTA TAAGAACAGAAC	900 910 920  PATC ACTANTANT TAACCOGGC ATAG TGATTANTA ATTGGGCCCG  1010 1020 1030  AACG GGAACAGGGT TTGTTGATTC FTGC CCTTGTCCCA AACAACTAAG  1120 1130 1140  GTAA GATGATGTTA ACTATGTGAT CATT CTACTACAAT TGATACCTA  1230 1240 1250  TCCT CAAAAAATAT ATTGGCATAT AGGA GTTTTTTATA TAAGCGTATA AAGGA GTTTTTTATA TAAGCGTATA 1340 1350 1360  GATA TAGTTTTTGA CACTATCTC CTAT ATCAAAAACT GTGATAGAAG  1450 1460 1470  CTGG ACATAATTCA TCTATTATAC GACC TGTATTAAAGT AGATAATATG  1560 1570 1580  TACA TGAAATGATC TCTATTGATG

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1590	1600	1610	1620	1630	1640	1650	1660	1670	1680 1	690
		TGAAAATTGG	TAACTCATTC	TATATATGCT	TICCTIGITG	ATGAAGGATA	GAATATACTC	AATAGAATTT	GTACCAACAA ACTGTTC CATGGTTGTT TGACAAG	
1700	1710	1720	1730	1740	1750	1760	1770	1780	1790 1	800
TATGAATCGT	ATATCATCAT	CTGAAATAAT	CATGTAAGGC	ATACATTTAA	CAATTAGAGA	CITGICICCT	GITATCAATA	TACTATTCTT	GTGATAATIT ATGTGTG CACTATTAAA TACACAG	
1810	1820	1830	1840	1850	1860	1870	1880	1890	1900 1	910
CAAATTTGTC	CACGITCITI	AATTITGITA	TAGTAGATAT	CARATCCART	GGAGCTACAG	TTCTTGGCTT	AAACAGATAT	AGTITITICIG	GAACAAATTC TACAACA CTTGTTTAAG ATGTTGT	ATTA
1920	1930	1940	1950	1960	1970	1980	1990	2000	2010 2	020
	CTTTGGGTAG	ATAACTCCCA	TGAAATCCTA	AATTAATTTA	TGCTATCGCA	TIGICCICGI	GCAAATATCC	AAACGCTITT	GTGATAGTAT GGCATTC CACTATCATA CCGTAAG	
2030	2040	2050	2060	2070	2080	2090	2100	2110	2120 2	130
GTCTAGAAAC CAGATCTTTG	GCTCTACGAA	TATCTGTGAC	AGATATCATC	TTTAGAGAAT	ATACTAGTCG	CGTTAATAGT	ACTACAATTT	GTATITITA	ATCTATCTCA ATAAAAA TAGATAGAGT TATTTTT	TAAT
2140	2150	2160	2170	2180	2190	2:	200	2210	2220 22	30
TAXTATCTAT	GATTCAATGT	ATAACTAAAC	TACTAACTGT	TATTGATAAC	TAGAATCA GA ATCTTAGT CT	NA TCT AAT ( FT AGA TTA ( F R I	BAT GAC OTA CTA CTG CAT I V Y	TGG TTC TTC	THE ATC TAC TGC C	AA TT L
		2250	7260	2270				•	2310 23	
	ATT ATT T	T AGC ATC 1	CG TT AGA	TTT TCC AT	TGC CTT A	re gaa tae '	TOT TOO GIC	GAT GTC TAC	2310 23	GT
AAA TCG ACC	TAA TAA A	UA TCG TAG A	AGC AAA TCT R K S	AAA AGG TAG	ACG GAA TI A K I	AG CTT ATG	ACEA AGG CAG R G D	CTA CAG ATO	TGT CCG TAT TIT A	CA
			•••••			E3L			·	•
2330	2340	2350	2360	) , 2:	370	2380	- 2390	2400	2410	
AGG AGA GTT	T ACT AGG CO	C AAC TGA 2	TC AAT ACG	AAA AGA CC	ATC TCT C	T AGT TAT	TIG GCA GIA	CTC ATT AAT	2410 AAT GGT GAC AGG G	T
TCC TCT CAR	S P	vs	EIR	₽ 5 W	DRI	C T I	QCY	GAG TAA TTI E N I	TTA CCA CTG TCC C	AA N
24	120	2430	2440	2450	2460	2-	470	2480	2490 25	00
AGC ATC TTT	CCA ATC AS	T AAT TIT T	MIT AGC CGG	AAT AAC AT	ATC AAA AC	A CIT ATG	ATC CTC TCT	CAT TGA TT	TITC GCG GGA TAC A	
			K A P	I V D		5 K H	D E R	N S K	ERSV	
25	510	2520	2530	2540	255	0 2	560	2570	2580 25	90
	GAC GTC AG	C CAT AGC A	ATC AGC ATC	CGG CTT ATO	2 <b>0</b> 500 CTC 00	ET TGT CAT	aaa cca acg	AGG AGG AAS	TATC GTC GGA GCT CA TAG CAG CCT CGA C	TA
<d i="" i<="" td=""><td>V D A</td><td>A M</td><td>DAD</td><td>PKD</td><td>A E ?</td><td>r T M</td><td>F W R</td><td>P P I</td><td>D D S S</td><td>Υ</td></d>	V D A	A M	DAD	PKD	A E ?	r T M	F W R	P P I	D D S S	Υ
26	500	2610	2620	2630	264	0 2	650	2660	2670 20	580
GTG GTA TOO	C ACT ACG 17 F TGA TGC AM	NG AAG ATC (	TA CAG AGC	AA TEA TEE	C TTC TCG C	PT CTC CAT AA GAG GTA	ATT AAG TTG TAA TTC AAC	AGA TCA AT	C TTG TGC AGC AGT A C AAC ACG TCG TCA T	AGC FCG
cA M Y	SRC	) L D	YLA	K N V	ERI	KEM	N L Q	R T L	QAAT	A
. 26		2700	2710	. 2720	273		740	2750	2760 2770	
AGG AAG CTZ	AGG TTA C	A AAA TTA T	TOT DOD DOT	GTG TTA GA	C TGC GTC AG G ACG CAG TG A D :	CT TGC GAG	CAG TTA TAT D I Y		R CAT IT TTAGAGAGAI I GTA AA AATCICICI	
2780	2790	2800	2810	2820				2860	2870	2880
									2870 CTGATTAACCC GTCATC	•
GATTGTGTTG 2890							•		ACTAATTGGG CAGTAG	
			2920	2930	2940	2950		2970		2990
									GTARCATTAA CATTGCC CATTGTAATT CTAACG	
3000	3010	3020	3030	30,40	3050	3060	3070	3080	3090	3100
	TTGGGAGGCT	TAAAGTGTTG	TTTGCAATCT	CTACACGCGT	GTCTAACTAG	TGGAGGTTCG	TCAGCTGCTC	TAGTTTGAAT	CATCATCGGC GTAGTA' GTAGTAGCCG CATCAT	TTCC

## 15/15

3110	3120	3130	3140	3150	3160	3170	3180	3190	3200	3210
			ATTTCTCGTC			TGTAACTCAC		TTATCTATAT AATAGATATA		
3220	3230	• •	• •	• •		• •				3320
								AAGTAAAATA TTCATTTTAT		
3330	3340	3350	3360	3370	3380	3390	3400	3410	3420	3430
								AAATGATACA TITACTATGT		
3440	3450	• •	3470						3530	
								ACTIATGGGT TGAATACCCA		AAAGATTCAT TTTCTAAGTA
3550	3560	• •		• •						• •
								TCAATTACTA		ATTGTTTAAG TAACAAATTC
3660	3670	3680	3690	3700	3710	3720	3730	3740	3750	3760
		• •								
								TAATACTATC		
3770	3780	3790		3810	3820		3840	3850	3860	3870
CIGITATATG	TATCAACAAT	ACAGGCAGAT	CIATGGTTAT	GGTAAAACAC	TGTAACGGGA	AGCAGCATTC	TATGGTAACT	GGCCTATGTT	TAATAGCCAG	ATCATTTTAC
CIGITATATG	TATCAACAAT	ACAGGCAGAT	CIATGGTTAT	GGTAAAACAC	TGTAACGGGA	AGCAGCATTC	TATGGTAACT	GGCCTATGTT	TAATAGCCAG	
CIGITATATG	TATCAACAAT	ACAGGCAGAT	CIATGGTTAT	GGTAAAACAC CCATTTIGTG	TGTAACGGA ACATTGCCCT	AGCAGCATTC TCGTCGTAAG	TATGGTAACT ATACCATTGA	GGCCTATGTT CCGGATACAA	TAATAGCCAG ATTATCGGTC	ATCATTTTAC
CTGTTATATG GACAATATAC 3880 TCTATAAACA	TATCAACAAT ATAGTIGITA 3890 TITTACCACA	ACAGGCAGAT TGTCCGTCTA 3900 AATAATAGGA	CTATGGTTAT GATACCAATA 3910 TCCTCTAGAT	GGTAAACAC CCATTITGTG 3920 ATTTAATATT	TGTAACGGA ACATTGCCCT 3930 ATATCTAACA	AGCAGCATTC TCGTCGTAAG 3940 ACAACAAAAA	TATGGTAACT ATACCATTGA 3950	GGCCTATGTT CCGGATACAA 3960 TGTATGGCCA	TAATAGCCAG ATTATCGGTC 3970 GAAGTATTTT	ATCATTITAC TAGTAANATG 3980 CTACTAATAN
CTGTTATATG GACAATATAC 3880 TCTATAAACA	TATCAACAAT ATAGTIGITA 3890 TITTACCACA	ACAGGCAGAT TGTCCGTCTA 3900 AATAATAGGA	CTATGGTTAT GATACCAATA 3910 TCCTCTAGAT	GGTAAACAC CCATTITGTG 3920 ATTTAATATT	TGTAACGGA ACATTGCCCT 3930 ATATCTAACA	AGCAGCATTC TCGTCGTAAG 3940 ACAACAAAAA	TATGGTAACT ATACCATTGA 3950	GGCCTATGTT CCGCATACAA	TAATAGCCAG ATTATCGGTC 3970 GAAGTATTTT	ATCATTITAC TAGTAANATG 3980 CTACTAATAN
CTGTTATATG GACAATATAC 3880 TCTATAAACA	TATCAACAAT ATAGTIGITA 3890 TITTACCACA	ACAGGCAGAT TGTCCGTCTA 3900 AATAATAGGA TTATTATCCT	CTATGGTTAT GATACCAATA 3910 TCCTCTAGAT AGGAGATCTA	GGTAAAACAC CCATTTTGTG 3920 ATTTAATATT TAAATTATAA	TGTAACGGGA ACATTGCCCT 3930 ATATCTAACA TATAGATTGT	AGCAGCATTC TCGTCGTAAG 3940 ACAACAAAAA TGTTGTTTTT	TATGGTAACT ATACCATTGA 3950 AATTTAACGA TTAAATTGCT	GGCCTATGTT CCGGATACAA 3960 TGTATGGCCA ACATACCGGT	TANTAGCCAG ATTATCGGTC 3970 GAAGTATTTT CTTCATAAAA	ATCATITIAC TAGTAAAATG 3980 CTACTAATAA GATGATTATT
CTGTTATATG GACAATATAC  3880 TCTATAAACA AGATATTTGT  3990 AGATAAAGAT	TATCAACAAT ATAGTIGITA  3890 TITTACCACA AAAATGGTGT  4000 AGTCTATCTT	ACAGGCAGAT TOTCCGTCTA 3900 AATAATAGGA TTATTATCCT 4010 ATCTACAAGA	CTATGGTTAT GATACCARTA 3910 TCCTCTAGAT AGGAGATCTA 4020 TATGAAAGAA	GGTAAACAC CCATTTGTG 3920 ATTTAATATT TAAATTATAA 4030 GATAATCATT	TGTAACGGA ACATTGCCCT  3930 ATATCTAACA TATAGATTGT  4040 TAGTAGTAGC	AGCAGCATTC TCGTCGTAAG 3940 ACAACAAAA TGTTGTTTT 4050 TACTAATATG	TATGGTAACT ATACCATTGA  3950 AATTTAACGA TTAAATTGCT  4060 GAAAGAAATG	GGCCTATGTA  3960 TGTATGGCCA ACATACCGGT  4070 TATACAAAAA	TANTAGCCAG ATTATCGGTC  3970 GAAGTATITT CTTCATARAA  4080 CCTGGAAGCT	ATCATITIAC TAGTAAAATG 3980 CTACTAATAA GATGATTATT
3880 TCTATAAACA AGATATTIGT 3990 AGATAAAGAT TCTATAAAGAT	TATCAACAAT ATAGTIGITA  3890 TITTACCACA AAAATGGTGT  4000 AGTCTATCTT	ACAGGCAGAT TOTCCGTCTA 3900 AATAATAGGA TTATTATCCT 4010 ATCTACAAGA	CTATGTAT GATACCANTA 3910 TCCTCTAGAT AGGAGATCTA 4020 TATGAAAGAA AIACTTTCTT	GGTAAAACAC CCATTTIGTG 3920 ATTIAATAT TAAATTATAA 4030 GATAATCATT CTATTAGTAA	TGTAACGGA ACATTGCCCT  3930 ATATCTAACA TATAGATTGT  4040 TAGTAGTAGC ATCATCATCG	AGCAGCATTC TCGTCGTAAG  3940 ACAACAAAAA TGTTGTTTT  4050 TACTAATATG	TATGGTAACT ATACCATIGA 3950 AATTTAACGA TTAAATTGCT 4060 GAAGGAAATG CTTTCTTTAC	GGCCTATGTT CCGGATACAA 3960 TGTATGGCCA ACATACCGGT 4070 TATACAAAA ATATGTTTT	TAATAGCCAG ATTATCGGTC 3970 GAAGTATITT CTICATAAAA 4080 CGTIGGAAGCT GCACCTICGA	ATCATTATA TAGTAAAATG  1980 CTACTAATAA GATGATTATT  4090 TITATATTAA AAATATAATT
CTGITATATG GACAATATAC 3880 TCTATAAACA AGATATTIGT 3990 AGATAAAGAT TCTATTICTA 4100	TATCAACAN ATAGTIGTIA 3890 TITTACCACA AAAATGGTGT 4000 AGTCTATCTT TCAGATAGAA 4110	ACAGGCAGAT TOTCCGTCTA 3900 AATAATAGGA TTATTATCCT 4010 ATCTACAAGA TAGATGTTCT 4120	CTATGGTTAT  3910  TCCTCTAGAT AGGAGATCTA  4020  TATGAAAGAA ATACTTTCTT	GGTAAAACAC CCATTTIGIG 3920 ATTTAATAT TAAATTATAA 4030 GATAATCATT CTATTAGTAA 4140	TGTALCGGA ACATTGCCCT  3930 AIATCTAACA TATAGATTGT  4040 TAGTAGTAGC ATCATCATCG	AGCAGCATTC TCGTCGTAAG 3940 ACAACAAAAA TGTTGTTTT 4050 TACTAATATG ATGATTATAC	TATGGTAACT ATACCATTGA  3950 AATTTAACGA TTAAATTGCT  4060 GAAAGAAATG CTTTCTTTAC	GGCCTATACAA  3960 TGTATGGCCA ACATACCGGT  4070 TATACAAAAA ATATGTTTTT  4180	TAATAGCCAG ATTATCGGTC  3970 GAAGTATITT CTTCATARAA  4080 CGTGGAAGCT GCACCTTCGA	ATCATTITAC TAGTAAAATG  3980 CTACTAATAA GATGAITATT  4090 TITATATTAA AAATATAATT  4200
3880 TCTATAAACA AGATATTIGT  3990 AGATAAAGAT TCTATTICTA  4100 ATAGCATATT	3890 TITTACCACA AAAATGGTGT  4000 AGTCTATCTT TCAGATAGAA ALL ALL ACTAGAAGAA	ACAGGCAGAT TOTCCOTCTA 3900 AATAATAGGA TTATTATCCT 4010 ATCTACAAGA TAGATGTTCT 4120 TTAAAATCTA	CATACCARTA  3910  TCCTCIAGAT AGGAGATCIA  4020  TATIGAAAGAA AIACTITCTT  4130  GACTIAGTAT	GGTAAAACAC CCATTTIGTG 3920 ATTIAATATT TARATTATAA 4030 GATAATCATT CTATTAGTAA 4140 AACCAAAACAG	TGTAACGGA ACATTGCCCT  3930 ATATCTAACA TATAGATTGT  4040 TAGTAGTAGC ATCATCATCG  4150 TTAAATGCCA	AGCAGCATTC TCGTCGTAAG  3940 ACAACAAAAA TGTTGTTTT  4050 TACTAATATG ATGATTATAC ATATCGATTC	TATGGTAACT ATACCATTGA 3950 AATTTAACGA TTAAATTGCT 4060 GAAAGAAATG CTTTCTTAC 4170 TATATTCAT	GGCCTATGTT CCGGATACAA  3960 TGTATGGCCA ACATACCGGT  4070 TATACAAAAA ATATGTTTT  4180 CATACCGTA	TAATAGCCAG ATTATCGGTC  3970 GAAGTATTIT CTTCATAAAA  4080 CGTGGAAGCT GCACCTTCGA  4190 GTACATTAAT	ATCATTATA TAGTAAAATG  3980 CTACTAATAA GATGAITATT  4090 TITATATTAA AAATATAATT  4200
CTUITATATU GACAATATACA AGATATTIGT  J990 AGATAAAGAT TCIATTCIA  4100 ATAGCATATT TATUGTATAA	3890 TITTACCACA AAAATGGTGT  4000 AGTCTATCTT TCAGATAGAA ALL ALL ACTAGAAGAA	ACAGGCAGAT TOTCCOTCEA 3900 AATAATAGGA TTATTATCCT 4010 ATCTACAAGA TAGATOTTCT 4120 TTAAAATCTA AATTTTAGAT	CTATGGTAT  3910  TCCTCTAGAT AGGAGATCTA  4020  TATGGAAAGAA ATACTTTCTT  4130  GACTTAGTAT CTGGAATCATA	GGTAAAACAC CCATTTIGTG 3920 ATTIAATATT TAAATTATAA 4030 GATAATCATT CTATTAGTAA 4140 AACAAAACAG TTGTTTTGTC	TGTAACGGA ACATTGCCCT  3930 ATATCTAACA TATAGATTGT  4040 TAGTAGTAGC ATCATCATCG  4150 TTAAATGCCA AATTTACCGT	AGCAGCATTC TCGTCGTAAG  3940 ACAACAAAAA TGTTGTTTT  4050 TACTAATATG ATGATTATAC  4160 ATATCGATTC TATAGCTAAG	TATGGTAACT ATACCATTGA 3950 AATTTAACGA TTAAATTGCT 4060 GAAAGAAATG CTTTCTTTAC 4170 TATATTTCAT ATATAAAGTA	GGCCTATGTT CCGCATACAA  3960 TGTATGGCCA ACATACCGGT  4070 TATACAAAAA ATATGTTTT  4180 CATAACAGTA GTATTGTCAT	TAATAGCCAG ATTATCGGTC  3970 GAAGTATTTT CTTCATAAAA  4080 CGTGGAAGCT GCACCTTCGA  4190 GTACATTAAT CATGTAATTA	ATCATTATA TAGTAAAATG  3980 CTACTAATAA GATGAITAIT  4090 TITATATAA AAATAAATT  4200 CAGTGATATA GTCACTATAT
CTGITATATG GACAATATAC  3880 TCTATAAACA AGATATITGI  3990 AGATAAAGAT TCTATTCTA  4100 ATAGCATATT TATCGTATAA	TATCAACANT ATAGTTCTTA  J890 TITTACCACA AAAATGGTGT  4000 AGTCTATCTT TCAGGATAGAA  4110 ACTAGAAGAT TGATCTTCTA	ACAGGCAGAT TOTCCOTCTA 3900 AATAATAGGA TTATTATCCT 4010 ATCTACAAGA TAGATGTTCT 4120 TTAAAATCTA AATTTTAGAT	CTATGGTAT  3910  TCCTCTAGAT AGGAGATCTA  4020  TATGAAAGAA ATACTITCTT  4130 GACTTAGTAT CTGAATCATA  4240	GGTAAAACAG 3920 ATTTAATATT TARATTATAA 4030 GATAATCATT CTATTAGTAA 4140 AACAAAACAG TTGTTTTGTC	TGTAACGGA ACATTGCCCT  3930 ATATCTAACA TATAGATTGT  4040 TAGTAGTAGC ATCATCATCG  4150 TTAAAATGCCA AATTTACGGT	AGCAGCATTC TCGTCGTAAG  3940 ACAACAAAAA TGTTGTTTT  4050 TACTAATATG ATGATTATAC  4160 ATATCGATTC TATAGCTAAG	TATGGTAACT ATACCATTGA 3950 AATTTAACGA TTAAATTGCT 4060 GAAAGAAATG CTTTCTTAC 4170 TATATTCAT ATATAAAGTA 4280	GGCCTATGTT CCGGATACAA  3960 TGTATGGCCA ACATACCGGT  4070 TATACAAAAA ATATGTTTT  4180 CATAACAGTA GTATTGTCAT	TAATAGCCAG ATTATCGGTC  3970 GAAGTATTTT CTTCATAAAA  4080 CGTTGGAAGCT GCACCTTCGA  4190 GTACATTAAT CATGTAATTA	ATCATTATA TAGTAAAATG  3980 CTACTAATAA GATGATTATT  4090 TITATATTAA AAATAAATT  4200 CAGTGATATA GTCACTATAT  4310
CTUTATATG GACAATATAC  3880 TCTATAAACA AGATATTGT  3990 AGATAAAGAT TCTATTCTA  4100 ATAGCATATT TATCGTATAA  4210 CTGAAACGAT	TATCAACAAT ATAGTIGITA  3890 TITTACCACA AAAATGGTGT  4000 AGTICTATCTT TCAGATAGAA  4110 ACTAGAAGAAT TGATCTTCTA  4220 CTACAGAACATC	ACAGGCAGAT TOTCCOTCEA  3900 AATAATAGGA TTATTATCCT  4010 ATCTACAAGA TAGATOTTCT  4120 TTAAAATCTA AATTTTAGAT  4230 AACTATGCCAA	CTATGGTTAT  3910  TCCTCTAGAT AGGAGATCTA  4020  TATGGAAGAA ATACTTTCTT  4130  GACTTAGTAT CTGAATCATA  4240	GGTAAAACAC CCATTITGTG  3920 ATTITAATATI TAAATTATAA 4030 GATAATCATT CTATTAGTAA 4140 AACAAAACAG TTGTTTTGTC  4250 ATATGCCCAAT	TGTAACGGA ACATTGCCCT  3930 ATATCTAACA TATAGATTGT  4040 TAGTAGTAGC ATCATCATCG  4150 TTAAATGCCA AATTTACGGT	AGCAGCATTC TCGTCGTAAG  3940 ACAACAAAAA TGTTGTTTT  4050 TACTAATATG ATGATTATAC  4160 ATATCGATTC TATAGCTAAG  4270 ATTTTAACTT	TATGGTAACT ATACCATTGA  3950 AATTTAACGA TTAAATTGCT  4060 GAAAGAAATG CTTCCTTTAC  4170 TATATTCAT ATATAAAGTA  4280 TAGAACTAAA	GGCCTATGTT CCGCATACAA  3960 IGTATGGCCA ACATACCGGT  4070 TATACAAAAA ATATGTTTT  4180 CATAACAGTA GTATTGTCAT  4290 ACGTTCTACC	TAATAGCCAG ATTATCGGTC  3970 GAAGTATITT CTTCATAAAA  4080 CGTGGAAGCT GCACCTTCGA  4190 GTACATTAAT ATTATTA	ATCATTATA TAGTAAAATG  3980 CTACTAATAA GATGAITAIT  4090 TITATATAA AAATAAATT  4200 CAGTGATATA GTCACTATAT
3880 TCTATAACA AGATATTIGT  3990 AGATAAAGAT TCTATTTCTA  4100 ATAGCATATT TATCGTATAAA  4210 CTGAAACGAT GACTTTGCTA	TATCAACAAT ATAGTIGITA  3890 TITTACCACA AAAATGGTGT  4000 AGTICTATCTT TCAGATAGAA  4110 ACTAGAAGAAT TGATCTTCTA  4220 CTACAGAACATC	ACAGGCAGAT TOTCCOTCEA  3900 AATAATAGGA TTATTATCCT  4010 ATCTACAAGA TAGATOTTCT  4120 TTAAAATCTA AATTTTAGAT  4230 AACTATGCCAA	CTATGSTAT GATACCARTA 3910 TCCTCTAGAT AGGAGATCTA 4020 TATGAAAGAA ATACTTTCTT 4130 GACTTAGTAT 4240 GGAATAAGCA CCTTATTCGT	GGTAANACAC CCATTTIGTG  3920 ATTTAATATT TAAATTATAA  4030 GATAATCATT CTATTAGTAA  4140 AACAANACAG TTGTTTIGTC  4250 ATATGCGATT TATACGGTTA	TGTAACGGA ACATTGCCCT  3930 ALATCTAACA TATAGATTGT  4040 TAGTAGTAGC ATCATCATCG  4150 TTAAATTGCCA AATTTACCGT  4260 TATGTCTAAT ATACAGATTA	AGCAGCATTC TCGTCGTAAG  3940 ACAACAAAAA TGTTGTTTT  4050 TACTAATATA ATGATTATAC ATATGGATTC TATAGCTAAG  4270 ATTTTAACTT TAAAATTGAA	TATGGTAACT ATACCATIGA  3950  AATTTAACGA TTAAATTGCT  4060 GAAAGAAATG CTTTCTTTAC  4170 TATATATCAT ATATAAAGTA  4280 TAGAACTAAA ATCTTGATTT	GGCCTATGTT CCGGATACAA  3960 TGTATGGCCA ACATACCGGT  4070 TATACAAAAA ATATGTTTT  4180 CATAACAGTA GTATGTCAT  4290 ACGTTCTACC TGCAAGATGG	TAATAGCCAG ATTATCGGTC  3970 GAAGTATITT CTTCATARAA  4080 CGTGGAAGCT GCACCTTCGA  4190 GTACATTAAT ATGGTARTTA  4300 AATACTARAAA TTATGATTIT	ATCATTTAC TAGTAANATG  3980 CTACTAATAA GATGATTATT  4090 TITATATTAA AAATATAATT  4200 CAGTGATATA GICACTATAT  4310 ATAGGATACG TATCCTATGC
3880 TCTATAACA AGATATTIGT  3990 AGATAAAGAT TCTATTTCTA  4100 ATAGCATATT TATCGTATAA  4210 CTGAAACGAT GACTITGCTA	TATCAACAAT ATAGTIGITA  3890 TITTACCACA AAAATGGTGT  4000 AGTCTATCTT TCAGATAGAA  4110 ACTAGAAGAT TGATCTICTA  4220 CTACAGACTC GATGTCTGAG	ACAGGCAGAT TOTCCOTCTA 3900 AATAATAGGA TTATTATCCT 4010 ATCTACAAGA TAGATOTTCT 4120 TTAAAATCTA AATTTTAGAT AATTTTAGAT 4230 AACTATCCAA TTGATACCAT	CTATGGTTAT  3910  TCCTCTAGAT AGGAGATCTA  4020  TATGAAAGAA ATACTTTCTT  4130  GACTTAGTAT CTGAATCATA  4240  GGAATAAGCA CCTTATTCGT	GGTAAAACAC CCATTITGTG  3920 ATTITAATATI TAAATTATAA 4030 GATAATCATT CTATTAGTAA 4140 AACAAAACAG TTGTTTTGTC  4250 ATATGCCAAT TATACGGTTA 4360	TGTAACGGA ACATTGCCCT  3930 ALATCTAACA TATAGATTGT  4040 TAGTAGTAGC ATCATCATCG  TTAAATGCCA AATTTACCGT  4260 TATGTCTAAT ATACAGATTA ATACAGATTA 4370	AGCAGCATTC TCGTCGTAAG  3940 ACAACAAAAA TGITGITTT  4050 TACTAATATA ATGATTATAC ATATCGATTC TATAGCTAAG  4270 ATTITAACTT TAAAATTGAA	TATGGTAACT ATACCATIGA  3950  AATTTAACGA TTAAATIGCT  4060 GAAAGAAATG CTTTCTTTAC  4170 TATATATCAT ATATAAAGTA  4280 TAGAACTAAA ATCTTGATTT  4390	GGCCTATGTT CCGCATACAA  3960 TGTATGGCCA ACATACCGGT  4070 TATACAAAAA ATATGTTTT  4180 CATAACAGTA GTATTGTCAT 4290 ACGTTCTACC TGCAAGATGG	TAATAGCCAG ATTATCGGTC  3970 GAAGTATTTT CTTCATAAAA 4030 CGTGGAACCT GCACCTTCGA 4190 GTACATTAAT CATGTAATTA 4300 AATACTAAAAA TTATGATTTT  4410	ATCATTTAC TAGTAAAATG  3980 CTACTAATAA GATGATTATT  4090 TTTATATTAA AAATATAAAT  4200 CAGTGATATA GTCACTATAT  4310 ATAGGATACG TATCCTATGC

4430 TITATGAAGG TACC AAATACTTCC ATGG